The yeasts and their chemical changes in the British fresh sausage.

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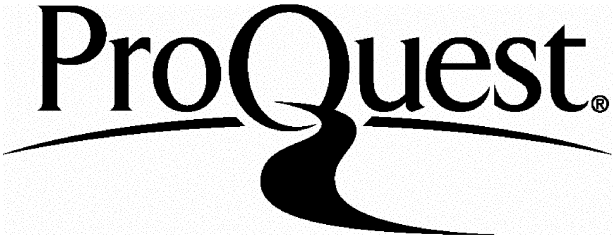
THE YEASTS AND THEIR CHEMICAL CHANGES IN THE BRITISH FRESH SAUSAGE

Submitted by Hilary Karen Dalton for the degree of Ph.D. of the University of Bath 1984.



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SUMMARY

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Seven hundred and seventeen yeasts isolated from samples of sulphited and unsulphited sausage, skinless sausage, minced beef, and ingredients intended for sausage manufacture, as well as air and equipment in a sausage factory were characterised in detail. Debaryomyces hansenii was the most commonly occurring yeast in the majority of samples of sausage and minced beef, followed by Candida zeylanoides and Pichia membranaefaciens. The presence of sulphite did not appear to influence the overall numbers or range of yeasts in sausage but did affect their relative proportions such that the incidence of D. hansenii was higher and that of certain Cryptoccoccus and Rhodotorula spp. was lower in samples containing the preservative. The heat treatment of skinless sausages during processing appeared to reduce the incidence of D. hansenii. The factory survey showed that the meat intended for sausage manufacture and also other ingredients as well as equipment harboured the same range of yeasts as were found in the finished product. A yeast flora dominated by Trichosporon cutaneum was isolated from pig carcasses immediately following slaughter. This flora was found to be confined largely to the equipment and air of the slaughter area and lairage.

In general, the yeast flora of sausage was non-fermentative but could assimilate a wide range of carbohydrates. Selected strains of C. curvata, C. lipolytica var. lipolytica, C. zeylanoides, Cr. albidus, D. hansenii, P. membranaefaciens and Torulopsis candia were shown to synthesise either extra cellular lipases and/or amylases but not proteases in broth culture.

Sulphite binding compounds, principally acetaldehyde, were produced in lab lemco broth (pH 7.0) containing sulphite (500 $\mu g\ g^{-1}$) during the exponential phase of growth of representative strains of *D. hansenii*, *C. zeylanoides*, *P. membranaefaciens* and *T. candida* but not *Cr. albidus* var. *albidus* and *Rh.rubra*. The extent of sulphite binding in minced pork-belly supplemented with sulphite (500 $\mu g\ g^{-1}$) was appreciably greater in samples inoculated with *D. hansenii* than in those inoculated with representatives of the other members of the microbial association, *Brochothrix thermosphacta*, *Lactobacillus* sp. and a pseudomonad. The content of sulphite binding agents in minced pork was found to be positively related ($r = 0.98$, 0.92 at 1 and 15°C respectively) to the size of the yeast population. The concentration of acetaldehyde in stored sausages obtained from a factory and also those obtained from retail outlets was correlated with the concentration of bound sulphite ($r = 0.89$). The rate and extent of sulphite binding and acetaldehyde production was fastest during the exponential phase of yeast growth in sausages.

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INTRODUCTION

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Until recently it was generally accepted that yeasts, although ubiquitous throughout the meat industry, were of limited importance in the spoilage of meat or meat products unless the growth of bacteria had been arrested in some way (Walker and Ayres, 1970). This may well be the case with British fresh sausage, a product that can contain up to 2.4×10^4 yeasts g^{-1} at the time of sale (Dowdell and Board, 1971). Immediately after manufacture, sulphite, the legally permitted preservative in British fresh sausages inhibits the growth of the nutritionally non-fastidious bacteria, such as pseudomonads (Banks and Board, 1982), that become dominant in the microflora developing during the chill storage of joints from a variety of animal carcasses as well as minced beef, providing oxygen is present (Kirsch et al., 1952; Ayres, 1960; Wolin et al., 1957; Gardner, 1965). This inhibition may favour the growth of yeasts simply through ensuring that they have adequate substrates. The subsequent, albeit limited, growth of pseudomonads in sausages has been associated with a diminution of the concentration of free sulphite (Banks and Board, 1981). It has been suggested (Brown, 1977) that yeasts are the principal cause of sulphite binding in British fresh sausage but the means whereby they do so have not been determined. Although these recent observations have posed commercially important questions about the possible role of yeasts in the spoilage of sausages, little headway would be possible without information about the

source, identity and physiological attributes of these organisms. These topics were considered in detail in the present study. In addition representative strains of the commonly occurring species of yeasts were studied with the objectives of establishing their potential to bind sulphite and of identifying the binding compounds.

LITERATURE REVIEW

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Composition and manufacture of pork and beef sausages

The British fresh sausage is a mixture of raw meat (principally pork or pork and beef), pig fat, rusks (a biscuit, or in a few cases, a bread-crumb), cooked rinds, spices, approved dyes (red 2G, erythrosine and carmine), sodium chloride, polyphosphates, water and legally permitted levels of either a sodium sulphite or metabisulphite preservative (450 μg sulphite g^{-1}, Anon, 1962). The recipes of the pork and pork and beef sausages used in this study are given in Table 1.

A brief account of the method of manufacture used in this study and the probable changes that occur at that time are pertinent to an understanding of an empirically derived process. All the ingredients, with the exception of the rusks, are mixed in a bowl chopper at chill temperature, which is achieved by adding the bulk of the water as ice. Rapid comminution destroys the structure of the meat and fat such that the myofibrillar proteins are extracted from the meat and unfold in the environment (Swift, 1965) created by sodium chloride and aided by a low temperature (Karmas, 1977). These enclose the fat globules thereby creating a pseudo-emulsion (Borchert et al.,1967). Indeed the slurry formed in the bowl chopper is frequently described by the trade as a"fat-in-water"emulsion despite the absence of the obvious structures and properties of such. The pH of the slurry is stabilised at or near neutrality by the addition of polyphosphates

Table 1. Recipe of pork sausages used in this study.

Ingredient	% (w/w) Sausage
Lean pork	26.4
Pork back fat	22.0
Ice/Water	19.3
Rusks	12.3
Pork belly meat	7.0
Pork head meat	7.0
Rinds	4.0
Seasoning	1.7
Polyphosphates	0.3

such as sodium hexa phosphate, thus restricting the loss of water and soluble components from meat fibres which occurs when meat reaches its isoelectric point (pH 5.5; Lawrie, 1978). Emulsification is also favoured as the number of fat particles is reduced (Gerrard 1976). It has been suggested that the coating of fat particles with protein is perhaps the major factor in ensuring the random distribution of finely chopped fat particles in a liquid phase (Acton and Saffle, 1972). Rusk, the final ingredient to be added, enhances flavour (Leads, 1979) and absorbs water from the meat phase in the "emulsion", an essential feature because it ensures a texture that allows sausage meat to be pumped into casings (sausage skins). The casings may be the sub mucosa of the small intestines of pigs or sheep or, more commonly today, tubes formed from reconstituted collagen. The latter was used in this study. The sausages are then wrapped in a transparent film (polyethylene) and kept at chill temperatures (ca 5°C) throughout distribution and display.

The skinless sausage is similar in composition to the standard fresh sausage described above. It differs in that the proteins at the periphery of the links (individual sausages in cellophane tubes) are coagulated by heat (ca 71°C for 2 min.). This creates an "exoskeleton" which ensures the maintenance of the form of the sausage when the cellophane is removed.

Continental sausages differ from the commodities discussed above in a number of ways. Many of the former are fermented (Jensen, 1949; Smith and Palumbo, 1983) with starter cultures, either Pediococcus cerevisiae or Staphylococcus carnosus, and allowed to ripen before sale. Drying or smoking procedures may also be used

to extend the shelf life (Anon, 1953). The British fresh sausage contains rusk (ca 12% w/v of sausage, Pearson, 1970) whereas starch - normally flour, often of potato origin - is added to continental type sausages where it acts as a binding agent (Dahl, 1958).

Sulphite

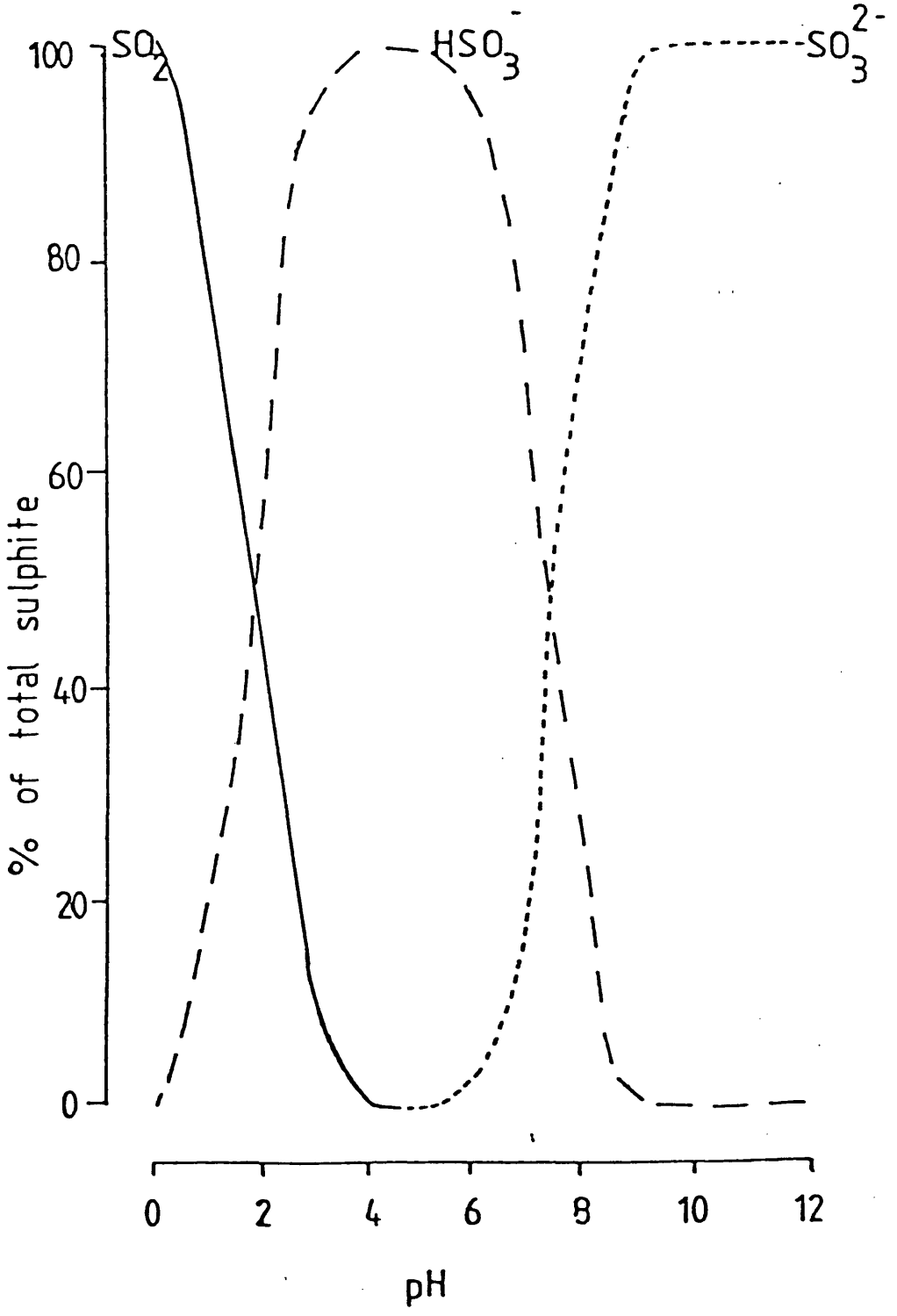
Sulphur dioxide and, to a lesser extent, its salts (sodium sulphite and sodium metabisulphite) have been used in a wide variety of foods and beverages as preservatives (Roberts and McWeeny, 1972), as antioxidants (e.g. potatoes Lund, 1968), or as inhibitors of enzymic and non-enzymic browning (Burton et al., 1963; Sullivan, 1971; Moussa, 1973). There appears to be no archival records relating to the adoption of sulphite or metabisulphite for the preservation of British fresh sausage. Indeed it was only about 20 years ago that the first evidence of the influence of these salts on the storage life of minced meat and sausage was published (Dyett and Shelley, 1962; Christian, 1963). Even today many in the trade consider sulphur dioxide to be the preservative agent. This situation is due in part to their failure to comprehend the role of pH in determining the ionisation of sulphite and in part to the methods used routinely to determine the concentration of preservatives in sausages - samples are boiled in mineral acid (Monier Williams', 1927, method modified by Shipton, 1959) - the sulphur dioxide in the distillate is trapped in hydrogen peroxide and titrated with sodium hydroxide.

Subsequent studies have shown that, in addition to their preservative role, these salts contribute to the texture, flavour and colour retention of the product (Leads, 1979).

Effect of pH on the antimicrobial activity of sulphite

The efficacy of the antimicrobial action of sulphur iv oxospecies are considered to be dependent on the degree of ionisation of the molecule (Douglas, 1966; Hammond and Carr, 1976). In dilute solution, three possible states of sulphurous acid exist namely :- molecular sulphur dioxide (SO_2), bisulphite ions (HSO_3^-) and sulphite ions (SO_3^{2-}) in a pH dependent equilibrium Indeed in the food industry, Vas and Ingram (1949) were probably the first to emphasize that the antimicrobial activity of sulphite was pH dependent. They used the first dissociation constant, k_1, of molecular SO_2 /HSO_3^- of 1.7×10^{-2} and that of the second dissociation constant k_2 of HSO_3^-/SO_3^{2-} of 5×10^6 to calculate the proportions of these molecular species in aqueous solutions at different pH values. Subsequent workers (King et al.,1981) have accepted the k_1 value used by Vas and Ingram (1949) but suggested that the latters' k_2 value was in error by 2 pH units from the more appropriate value of 6.31×10^{-8}. The efficacy of the antimicrobial activity of the three molecular species of sulphite is considered to decrease in the order $SO_2 > HSO_3^- > SO_3^{2-}$. Thus the calculations of (King et al., 1981; Figure 1) demonstrate that sulphite is most effective as a preservative in acidic products such as cider (Burroughs and

Figure 1 The percentage distribution of molecular SO_2, SO_3^{2-} and HSO_3^- in aqueous solution*.



* Adapted from King et al. (1981)

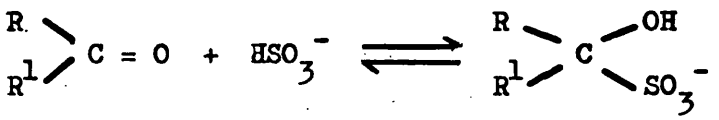
Sparks, 1964), orange juice (Ingram and Vas, 1950 a, b), jams (Dennis and Bugahair, 1980) and wines (King et al.,1981) in which the most antimicrobial moiety, molecular SO_2, is at a maximum concentration. In contrast, the pH of sausage (6.8 - 6.2) is such that the concentration of molecular SO_2 is of little practical significance and the active antimicrobial agents are sulphite and bisulphite ions (Hammond and Carr, 1976).

The fate of sulphite on storage

The concentrations of sulphite and bisulphite ions in the British fresh sausage diminish with storage (Brown, 1977). Anderton and Locke (1956), for example, showed that the total concentrations of sulphite and bisulphite ions decreased by ca 20% during the mixing of ingredients and that losses continued throughout storage. Brown (1977), who used an ion selective electrode in his studies, attributed this irretrievable loss to the oxidation of some of the sulphite and bisulphite ions to sulphate. He surmised that the reversible binding of sulphite and bisulphite ions to compounds of meat and microbial origin was the cause of the diminishing concentration of the free preservative of stored sausages. As only free sulphite and bisulphite ions are considered to have antimicrobial activity (Neuberg, 1929; Ingram, 1948; Rehm, 1964), the preservative potential of a sausage thus declines during storage. A better understanding of the extent of sulphite binding in sausages as well as of the concomitant reduction in the efficacy of the antimicrobial activity was not possible until a modified steam

distillation technique permitted the determination of free, bound and total sulphite (Banks and Board, 1982a; Banks, 1983). Thus Banks (1983) found that 26% of the sulphite was lost irretrievably during manufacture and a further 23% was bound. On subsequent storage at 4 or 22°C the concentrations of free sulphite in sausages decreased more rapidly than that of total sulphite, the rate of loss of both was greatest in sausages stored at room (22°C) rather than chill (5°C)temperatures. These observations, as well as those of Hammond and Carr (1976), indicate that the reversible loss of sulphite by binding - and hence impairment of sausage preservation - is of greater importance than that due to oxidation of the preservative via a free radical (Fridovitch and Handler, 1961) catalyst-sensitive chain reaction (Abel, 1913; Schroeter, 1966). It is for this reason that the emphasis in this review is given to the literature dealing with the reversible loss (binding) of sulphite in foods and microbial culture.

The binding of sulphite has been studied extensively (e.g. Ponting and Johnson, 1945; Ingram, 1948; Richardson, 1970), and a correlation is presumed to exist between the level of sulphite binding and the concentration of "soluble solids" in foods and beverages (Joslyn and Braverman, 1954). Sulphite, an extremely reactive molecule (Joslyn and Braverman, 1954), readily combines with a wide range of compounds in foods and beverages (Schroeter,



1966; Burroughs and Sparks, 1964, 1973). The reactions, which are considered rarely, if ever, to reach completion, with primarily carbonyls (→ α hydroxy sulphonates; Burroughs and Sparks, 1964, 1973), amines (→ amine bisulphites; Joslyn and Braverman, 1954), and sugars (→ sugar bisulphites; Ingram and Vas, 1950, a,b), are governed by dynamic reversible equilibria (Burroughs and Sparks, 1973), in which the equilibrium constant k is controlled by the equation :-

$$k = \frac{[S]\ [X]-[\underline{X}]}{[\underline{X}]}$$

Where [S] is the concentration of free sulphite, [X] is the concentration of binding agent and [$\underline{X}$] is the concentration of an undissociated addition compound. Such equilibria are dependent on pH and the concentration of sulphite but not the concentration of the binding agent. Ingram (1948) suggested that the rate and final equilibrium of a sulphite-binding reaction was a function of the temperature of storage or incubation. It is generally accepted that compounds whose equilibria are poised towards completion bind a higher proportion of the available sulphite than do those with lower affinities. Glucose, for example, has been shown to react more slowly than carbonyl compounds such as acetaldehyde with sulphite (Kerp, 1904). The study of binding equilibria of compounds isolated from cider (Burroughs and Sparks, 1964) led to the conclusion that (1) the reaction of sulphite with aldehydes was faster than that with ketones, and (2) that smaller carbonyl compounds reacted faster than larger ones. Indeed, the binding

equilibria for acetaldehyde was reported to be the fastest of the compounds recovered and it was suggested that it scavenged sulphite from other binding compounds.

The sulphite binding potential of foods and beverages, especially wines and cider (Kielhofer and Würdig, 1960; Burroughs and Sparks, 1964; Dittrich et al., 1973) has been shown to be enhanced by ingredients that have been physically damaged or heavily contaminated. Storage has also been shown to lead to an increase in the binding potential in cider (Burroughs and Sparks, 1973) and sausage (Banks, 1983). Burroughs and Sparks (1973) isolated 11 potential sulphite-binding compounds of microbial origin from cider. The principal ones were acetaldehyde, pyruvate, 2 oxoglutarate xylosone and galacturonic acid. Their concentrations were found to increase in the presence of sulphite. These workers estimated that the compounds noted above accounted for most of the sulphite-binding potential of cider. Likewise, experiments made on a sausage slurry revealed that the most extensive binding was associated with the microbial colonies rather than with the meat particles (Brown, 1977). As the extent of binding in sausages is reduced by chilled storage (Banks, 1983), it has been proposed that microbial growth rate and synthesis of sulphite binding compounds are related. Moreover the literature suggests that the presence of sulphite in foods and beverages stimulates components of the microbial flora to produce sulphite-binding compounds. As a consequence the efficacy of the preservative is reduced. The possible selective action of sulphite,

which will be discussed in more detail (pp 14-17), may in fact select organisms capable of producing sulphite binding compounds. This is considered to be the case in sausage (Banks, 1983) and acetaldehyde of yeast origin has been assumed to be the main binding agent (Brown, 1977). The ability of yeasts to produce sulphite-binding compounds has been reported repeatedly (e.g. Neuberg and Nord, 1929; Rankine and Pocock, 1969). It is generally accepted that the fermentative growth of yeasts changes the binding equilibria of a culture. For example, the fermentation of sugars, which have low affinities, gives products such as acetaldehyde, pyruvate and 2 oxoglutarate having high affinities for sulphite (Weeks, 1969; Graham, 1979). Moreover, Stratford (1983) noted that the secretion of binding compounds, principally acetaldehyde, into a culture medium by Saccharomycodes ludwigii was a feature of sulphited cultures only and that secretion ceased when all of the free sulphite had been bound. He concluded that the concentrations of acetaldehyde achieved in sulphited cultures were too large to be considered as cell reserves and suggested that acetaldehyde synthesis was induced by sulphite. Banks (1983) attributed the extensive loss of free sulphite from yeast-dominated sausages to the production of binding compounds by these organisms. The production of unidentified sulphite-binding compounds by yeasts isolated from sausage have been demonstrated (Brown, 1977; Banks, 1983) but the influence of yeast growth on the sulphite binding potential of sausage and minced meat has not been determined. It was in the present study (pp 134-183).

The selective action of sulphite

There is a wealth of literature concerning the preservation of acidic foodstuffs by molecular sulphur dioxide (Hammond and Carr, 1976; Carr, 1981). In view of the objectives of the present study only the literature concerning the influence of sulphite on the microbiology of neutral pH meat products will be reviewed in detail.

The earliest microbiological investigations of sulphited (300 mg / 10^6) raw minced meat (Lafontaine, 1955) and meat balls (Krol and Moerman, 1959) indicated that the addition of sulphite inhibited microbial growth at the outset of storage and storage at chill temperature severely retarded yeast growth. The enterobacteria in raw mince meat and in Saucisson (Fournaud et al., 1971) were considered to be particularly sensitive to growth inhibition by sulphite. Similar observations were made by Dyett and Shelley (1962, 1966), who examined British fresh sausages, and concluded that sulphite inhibited the growth of "coli-aerogenes" and other Gram-negative bacteria at incubation temperatures below 22°C and that it retarded the growth of the Gram-positive organisms as well. Christian (1963) noted a 2 - 3 fold extension of shelf life when sulphite (ca 2 - 3 grains / lb) was added to minced beef and he provided the first evidence that the preservative elected a spoilage (souring) flora dominated by Gram-positive bacteria. A Gram- negative flora caused putrefactive spoilage of the unsulphited minced beef. The addition of sulphite to vacuum packed bacon burgers extended the shelf life of the product by 2, 10 and 28d at 22, 10 and 5°C

respectively (Gardner, 1968). In this instance the composition of the microbial association did not appear to be altered but sulphite, perhaps in collaboration with nitrate and nitrite (Tompkin et al.,1980), appeared to retard the rate of growth of the dominant micro-organisms.

A survey of British fresh sausages from retail outlets (Dowdell and Board, 1967, 1968, 1971) revealed that a microbial association dominated by Brochothrix thermosphacta, yeasts and lactobacilli usually developed during storage and that the growth of the Gram-negative aerobic bacilli was retarded. These workers reported that the ability of isolates from sausages to grow in sulphited media was in the order :- yeasts > Br. thermosphacta ≥ lactobacilli > Pseudomonas spp. and coliforms. Ashworth et al.,(1974) confirmed that the addition of sulphite to sausage led to the development of the microbial association reported by Dowdell and Board (1971) and Tyson (1976) observed that the inhibition of growth of pseudomonads in sulphited minced meat was released once the concentration of free sulphite had fallen below a critical value. In keeping with the observations of Ashworth et al.(1974) she noted that the addition of polyphosphates enhanced the antimicrobial action of sulphite. Brown (1977), who studied in detail the effect of sulphite on the microbial association of sausage, concluded that the growth of the major microbial contaminants was not significantly affected by the legally permitted levels of sulphite (450 $\mu g\ g^{-1}$). Indeed, the yeasts in sausages appeared to be the least sensitive to elevated

concentrations of sulphite (one commercial brand of sausage contained ca 700 μg g^{-1} at the time of manufacture). In contrast he found that the Gram-negative mesophiles were the most sulphite-sensitive members of the initial contaminants of meat particularly at chill storage temperatures. This evidence, as well as observations made on materials obtained from a large factory, led Banks and Board (1981) to conclude that sulphite in sausages acts primarily as a selective agent rather than as a preservative sensu strictu. Indeed, Banks (1983) related the relative abundance of micro-organisms in stored sausages to their tolerance of free sulphite in culture media viz. (free sulphite concentration in parenthesis) : yeasts ($\geqslant$500 μg l^{-1}) Br. thermosphacta (470 - 500 μg g^{-1}) lactobacilli (250 - 400 μg g^{-1}); streptococci (310 - 410 μg g^{-1}); pseudomonads (16 - 330 μg g^{-1}) and enterobacteria (15 - 270 μg g^{-1}). The selective action of sulphite was emphasised by the behaviour of Ps. fragi in preserved sausages; this organism, the dominant member of the spoilage flora of chilled prime joints of meat (Molin and Ternstrom, 1982; Shaw and Latty, 1982), remained quiescent until the free sulphite content had declined to 110 μg g^{-1}. Another index of sulphite's selective action was provided by the members of the Enterobacteriacea. Enterobacter cloacea, Escherichia coli and Yersinia spp. were isolated frequently from sulphited sausages whereas Enterobacter agglomerans and 1 biotype of Hafnia alvei were isolated frequently from unsulphited ones. As the yeasts of sausages have not been characterised in detail, Banks (1983) was unable to study thoroughly the influence of

sulphite on the growth of these organisms. His observations that yeasts are tolerant to relatively high concentrations of sulphite is in general agreement with those of other workers (Robson, 1968; Roberts and McWeeny, 1972; Brown, 1977; Baird-Parker, 1980). Even allowing for adaptation to sulphite tolerance in yeasts (Robson, 1968), the available evidence supports the view that this attribute is associated with the mode of action on carbohydrate, those with a fermentative are more resistant than those with an oxidative metabolism (Rehm and Wittman, 1962; Reed and Peppler, 1973). The objective of the present study was an assessment of the elective action, if any, of sulphite on the yeast flora of meat and the sausages produced therefrom. In the course of the studies it was noted that sulphite was bound by yeasts considered to have an oxidative metabolism. This feature contrasts with the accepted views on sulphite binding - viz. binding compounds formed from carbohydrate fermentation (Rehm and Wittman, 1962; Reed and Pepler, 1973), and led (pp 202-205 of discussion) to a novel system being proposed.

Substrate availability

It is reasonable to assume that many diverse chemical changes occur in chilled sausages because enzymes will be released from the finely chopped meat and the ultimate mixing of ingredients may well ensure that substrates exhausted during the storage of meat are replenished by non-meat ingredients. It is well known that mincing enhances the chemical activity of meat as indexed by the

rate of glucose breakdown (Newbold and Scopes, 1971). The relatively large initial level of micro-organisms in fresh sausages (pp 31-35) would also contribute to the pool of enzymes dispersed throughout sausage meat. In practice, the breakdown of starch in rusk has been the sole study to date, mainly because of the conviction in the trade that sausages spoil through souring and that microbial fermentation of carbohydrates plays a key role.

The British fresh sausage contains a large amount of starch (Pearson, 1970; Abbiss,1978). The sources of starch in sausage are rusk, a biscuit crumb made from wheat flour (ca 800 mg insoluble carbohydrate g^{-1}), and lean pork (ca 2.2 mg insoluble carbohydrate g^{-1}). Dowdell and Board (1971) demonstrated the presence of reducing sugars throughout storage of sausages, even though there was a progressive increase in lactic acid presumably as a consequence of microbial fermentation. Abbiss (1978) noted that substrate concentrations of glucose, maltose and maltotriose (ca 7, 6 and 3 mg g^{-1} respectively) were maintained throughout the storage of pork sausages at 4 or 20°C. The studies of Dowdell and Board (1971) revealed also that sausages contained only 100 amylolytic organisms g^{-1}. These observations lead to the assumption that the microbial flora relied on the activity of meat enzymes for the release of substrates. As fats constitute 10 - 15% of the sausage, they represent a large reservoir of carbon and energy in the form of triglycerides. Valerate accumulation on storage of sausages suggest that lipids

are used as substrates for microbial growth (Abbiss, 1978). Glycerol, potentially an important energy-yielding substrate in sausage, is considered to be released from triglycerides by glycerol esterhydrolases of meat and microbial origin (Pearson, 1970). The enzymes of yeasts are thought to be involved also (Leads, 1979). Extensive protein reserves of meat (22.4 mg protein g^{-1}) and rusk (8.8 mg protein g^{-1}) origin are present in sausages but the findings of Mossel and Ingram (1955) suggests that the high concentrations of carbohydrate would exert a sparing effect on protein utilisation by encouraging the production of an acidic pH by carbohydrate metabolism. It may be presumed, however, from the pH of the sausage, which is ca 6.2 - 6.8 at the point of manufacture and varies no more that 1 pH unit towards acidity following storage (Dowdell and Board, 1971), that this situation would not occur in sausages.

Microbiology

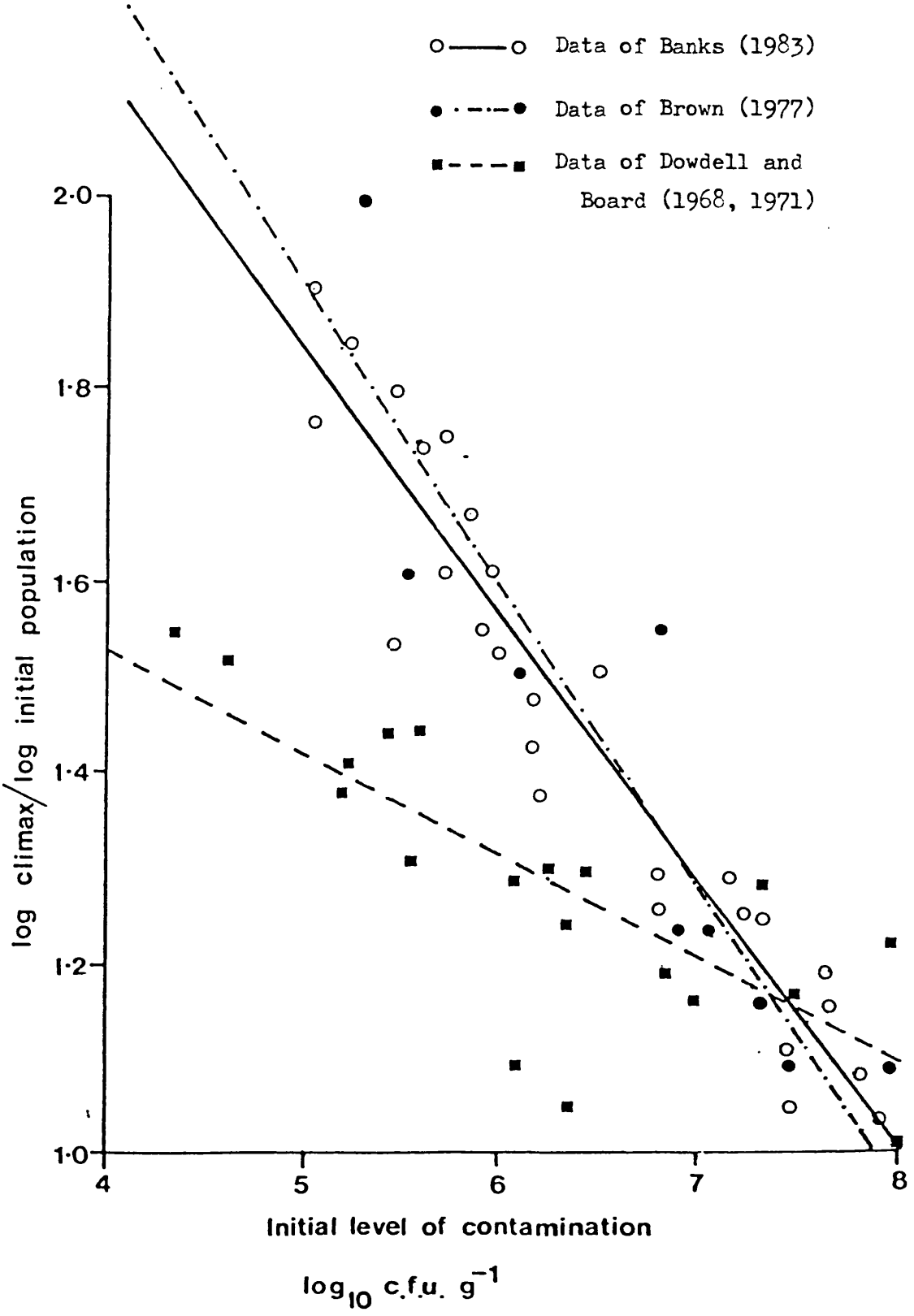
Source of microbial contaminants in sausage

The occurrence of a microbial association of Br. thermosphacta, yeasts, lactobacilli and micrococci in sausages is well established (Dowdell and Board, 1971; Brown, 1977; Abbiss, 1978; Leads, 1979). The rate and extent of the development of the association is related to the size and composition of the initial infection (Figure 2, Banks, 1983). As pork and beef, produced under accepted conditions of good manufacturing practice, harbour the dominant microbial contaminants of sausage (Ayres, 1960; Dowdell and Board, 1971), the literature dealing with the sources of contaminants of meat will be considered.

Nottingham (1982) was of the opinion that three factors influence the microbial quality of meat: (1) the state of the animal at the time of slaughter; (2) the spread of contamination during slaughter, and (3) the temperature of and the time taken to process a carcass.

It is generally accepted that there are two sources of contaminants to which meat is exposed : (1) intrinsic infections from organisms resident in the gut or on mucous membranes and (2) extrinsic infection from contaminants present in abattoirs and on equipment used for butchering a carcass. The organisms from the first mentioned depots are considered to infect meat as a result of penetrating the gut and mucous membranes (Wilson and Miles, 1964)

Figure 2 Regression lines describing the influence of the initial level of microbial contamination on the final climax population size in sausage*.



* Adapted from Banks (1983)

and subsequent dissemination *via* the lymphatic system (Gill, 1979). More invasive organisms, which are found in compromised or infected hosts, may provide a source of persistent or recurrent infections of the tissues (Von Graevenitz, 1977). As these organisms are predominantly mesophilic, they grow and cause spoilage only when there is a sluggish loss of heat from a carcass. Bone taints, for example, arising from the growth of *Clostridium* spp. was a problem in hams before the general use of refrigerated stores in large abattoirs (Callow and Ingram, 1955). Although the gut and mucous membranes would, at times, harbour pathogenic micro-organisms, veterinary inspection during slaughter precludes the transfer of such organisms to man. Nevertheless, the high incidence of recovery of *Salmonella* spp., albeit small in numbers, from the lymph nodes associated with the viscera suggests a transfer of pathogens in animals deemed to have been healthy at the point of slaughter (Smith, 1959; Nottingham and Urselman, 1961; Robinson, 1970, Nottingham, *et al*., 1972, Nazer and Osborne, 1976). Indeed, this may be an important avenue of infection of sausages. Several surveys of this commodity have shown incidences of *Salmonella* contamination of 65% of pork and 55% of pork and beef sausages (Banks, 1983; Banks and Board, 1983). Moreover, the extreme sulphite sensitivity of these organisms (Banks and Board, 1982b) probably accounts for the very low level of salmonellosis associated with sausages (10 outbreaks during the period from 1967 - 1972, Anon, 1975). The assessments of the extent of intrinsic contamination of healthy

carcasses with non-pathogenic organisms are discordant. Some workers (Reith, 1929; Jensen and Hess, 1941; Vanderzant and Nichelson, 1969; Zagaevskii,1973) have apparently succeeded in isolating organisms from the tissues of freshly slaughtered animals but others (Radouco - Thomas et al.,1959; Hasegawa et al.,1970; Buckley et al.,1976; Gill et al, 1978; Gill,1979, 1980)have not. From a critical review of the literature, Gill (1979) deduced that many of the claims of 'successful' isolation may have been associated with inadequate asepsis at the time of sampling, a serious technical problem when very low numbers of commonly occurring organisms are sought. Oral administration of indicator organisms to animals immediately before slaughter have been adopted in studies of intrinsic infection in an attempt to overcome this problem. Thus Mackey and Derrick (1979) isolated Escherichia coli ABR 2 from lungs and spleens of pigs infected orally with this organism. Labadie et al.(1977), who injected pigs intravenously with Enterococcus ("Streptococcus") faecium and Pseudomonas fluorescens, recovered these organisms from the liver, spleen and mesenteric ganglia. In both cases, the organisms were not recovered from the blood and muscles. Gill (1979) attributed the absence of organisms in tissue fluids to the inability of the resident organisms of healthy animals to resist the antibacterial agents of the blood. When reviewing the available literature Roberts (1980) concluded that, even if there were a high incidence of intrinsic contamination of meat, the low levels of contamination was of no importance

commercially. Indeed, he stressed that extrinsic contamination was the major source of microbial contamination of meat.

The discussion of extrinsic contamination will be considered in the context of the large factory in which my work was done (Figure 3). After an overnight stay in the lairage the pigs were electrically stunned and their throats cut ("stuck"). The dead pigs were passed through a scalding tank ($60^{\circ}C$ for 6 minutes), to loosen the hair follicles, dehaired mechanically and singed in a furnace (700° - $900^{\circ}C$) for 15 s to carbonise the outer layer of the epidermis which was removed by rollers. The carcasses were then eviscerated, split along the backbone, weighed and put in a cold store (ca $5^{\circ}C$). On the following day, the carcasses were butchered and the cuts - head, bellymeat, back fat and rind - intended for sausage production, were stored overnight before comminution with other ingredients in a bowl chopper.

The surface of pigs at the time of slaughter are heavily contaminated - 1×10^5 - 1×10^6 organisms g^{-1} (Jepson, 1947; Snijders and Gerats, 1976). Indeed, the hides, rumen, soil, faeces and air in abattoirs have been shown to contain massive populations of bacteria and to a lesser extent yeasts and moulds of which a small proportion were psychrotrophic (Table 2, Empey and Scott, 1939). The findings of Newton et al. (1978) confirmed these observations and demonstrated that the psychrotrophic counts were influenced by seasonal variations (Table 3). The stick knife and the act of sticking have been considered to be a major cause of contamination of meat because both the knife (Gerrard and

Figure 3 Flow diagram of the manufacturing proce dures in a sausage factory with its own abattoir.

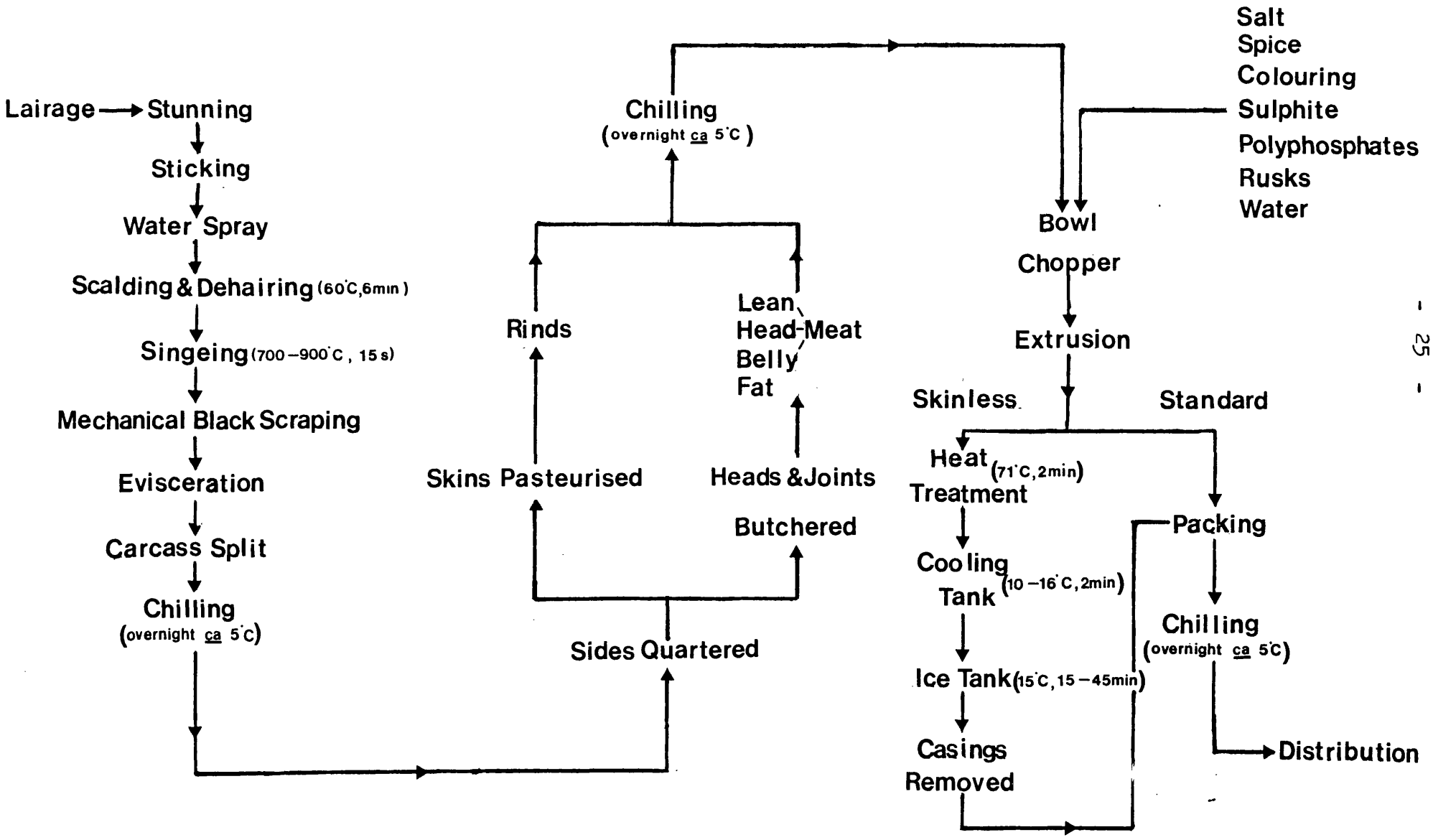


Table 2 Typical counts of microbial contamination in an abattoir

Sources of contamination	Incubation Temperature (°C)	Bacteria	Yeasts	Moulds
Hides (cm^{-2})	20	3.3×10^6	5.8×10^2	8.5×10^2
	-1	1.5×10^4	8.9×10^1	8.9×10^1
Surface soils (g^{-1} dry weight)	20	1.1×10^8	5.0×10^4	1.2×10^5
	-1	2.8×10^6	1.4×10^4	1.0×10^4
Faeces (g^{-1} dry weight)	20	9.0×10^7	2.0×10^5	6.0×10^4
	-1	2.0×10^5	7.0×10^1	1.7×10^3
Rumen (g^{-1} dry weight)	20	5.3×10^7	1.8×10^5	1.6×10^3
	-1	5.2×10^4	5.0×10^1	6.0×10^1
Air (no deposited $cm^{-2}h^{-1}$)	20	1.4×10^2	-	2.0×10^1
	-1	8.0×10^1	-	1.0×10^{-1}

Adapted from Empey & Scott (1939)

Table 3 Microbial counts on hides, walls, floors and drains in an abattoir ($\log_{10}$ cm $^{-2}$).

		Hides		Walls				Floors and drains			
				1		2		1		2	
		T	P	T	P	T	P	T	P	T	P
Summer	$\bar{x}$	3.80	L	2.82	0.83	3.50	L	5.36	0.84	4.36	L
	s	0.27	0.58	0.72	0.75	0.86	0.54	0.27	1.34	0.68	0.58
	n	5	5	3	3	4	4	4	4	5	5
Autumn	$\bar{x}$	4.28	1.87	nr	nr	nr	nr	nr	nr	nr	nr
	s	0.46	1.37								
	n	7	7								
Winter	$\bar{x}$	4.62	2.54	3.73	2.09	2.59	0.12	5.50	3.30	5.80	2.0
	s	0.55	0.96	0.80	1.29	1.44	1.10	0.34	0.95	0.81	1.38
	n	11	11	12	12	10	10	9	9	12	12
Spring	$\bar{x}$	nr	nr	2.92	1.50	3.24	0.28	5.63	2.18	4.78	0.44
	s			0.28	1.18	1.74	0.63	0.27	1.70	0.62	1.15
	n			5	5	7	7	5	5	8	8
Combined	$\bar{x}$	4.34	1.78	3.39	1.75	2.98	0.14	5.57	2.44	5.24	1.09
Counts	% P/T	0.27		2.29		0.14		0.07		0.01	
"t"		5.35		1.61		0.27		3.81		3.15	

continued

Table 3 continued

1	chilled areas
2	unchilled areas
T	total counts
P	psychrotrophic counts
$\bar{x}$	mean counts
s	standard deviation
n	number of determinations
"t"	students "t" test comparison of summer v winter psychrotrophic counts
L	<1 cell cm $^{-2}$
nr	not recorded

Adapted from Newton et al. (1978)

Mallion, 1977) and the skin surrounding the wound in the neck can be heavily contaminated (ca 10^4 - 10^6 and 10^5 - 10^9 organisms cm^{-2}, respectively). Indeed, experiments made with sticking knives deliberately contaminated with known bacteria have shown that organisms spread to the bone marrow (Jensen and Hess, 1941) and other organs with phagocytic activities (Mackey and Derrick, 1979; Labadie et al., 1977) during the short period of blood circulation following sticking. The microbial contamination of the surface of slaughtered pigs, especially with enterobacteria (Gerats et al, 1981), is diminished (0.9 - 2.5 log cycles) during scalding (Dockerty et al., 1970; Snijders, 1976; Snijders and Gerats, 1976). The effectiveness of scalding is considered to be proportional to the temperature used, the optimum kill being achieved with high temperatures and alkaline water for a short period of time (60°C, 7 minutes, Dockerty et al., 1970). Indeed a reduction in the bacterial count by 99.7% has been reported (Dockerty et al., 1970). The time and temperature of scalding (60°C for 6 minutes) are such, however, that organisms in the hair follicles, for example, are protected (Butler et al., 1980) and Roberts (1980) considered that, through the accumulation of debris from carcasses, the scalding tank may even serve as a depot of infection. Thus, the lungs of pigs have been shown to acquire bacteria from this source (Jensen and Hess, 1941). Indeed, Jones et al. (1979) demonstrated "contamination" of the tissues of pigs which had been submerged in a scald tank containing radioactive tracers. As the temperature of the carcass is far lower than the

surrounding scald water, actual growth of the bacteria on the carcass may occur (Nottingham, 1982). Although the singeing process (700° - 900°C) might be expected to kill large numbers of micro-organisms, the efficacy of this process evidently varies from plant to plant. Thus, both small (< 1 log cycle: Dockerty et al., 1970; Butler et al., 1980) and large ca 2.5 - 3 log cycles: Snijders, 1976; Snijders and Gerats, 1976; Rashe et al., 1978) reductions in the level of carcass contamination have been reported. Nevertheless, the level of microbial contamination of pig carcasses is considered to be at its lowest level immediately following singeing (Gardner, 1982) and subsequent procedures tend to cause recontamination (Snijders and Gerats, 1976). Thus, mechanical scraping for the removal of the carbonised material on the singed carcasses can be the cause of heavy recontamination (Gerats et al., 1981) and the standards of hygiene during evisceration will determine the ultimate level of contamination (Gardner, 1982; Roberts, 1980), even when evisceration is achieved without the gut being punctured (Gerats et al., 1981). The implements and hands of workers will transfer organisms to the inner and cut surfaces of the carcasses (Dockerty et al., 1970). Overnight storage at ca 5°C and high humidity can have a profound effect on the microflora of the carcass (Buthaux and Catsaras, 1966) because the conditions favour the growth of Gram-negative aerobic psychrotrophic bacilli such as pseudomonads that spoil chilled joints of meat or meat products (Gill, 1979). Subsequent butchering of a carcass can cause further contamination especially when the

knives and chopping boards are improperly cleaned, the working practices of butchers are slovenly and the temperatures of the butchery is uncontrolled, (Gardner, 1973; Gill, 1979). In practice, a common microbial flora is present on the workers hands, equipment as well as on walls and air throughout the factory (Empey and Scott, 1939). It contains pseudomonads, Br. thermosphacta, Gram-positive rods, yeasts and enterobacteria (Patterson and Gibbs, 1980) the principal contaminants of meat (Ayres, 1960) and sausage (Dowdell and Board, 1967) at the point of manufacture.

The microbial association of the British fresh sausage

The earliest microbiological investigations of sulphited British fresh sausage, both pork and beef, were those of Dyett and Shelley(1962, 1966) who demonstrated the development, after an initial lag period of 23 - 36 h, of a flora dominated by Gram-positive bacteria which they identified with Bacillus, Micrococcus and Streptococcus. A population of Gram-negative bacteria was found in unsulphited sausages. Their failure to isolate Br. thermosphacta has been attributed to the incubation of plate count agar (PCA) at too high a temperature, 30°C (Banks, 1983). The subsequent work of Dowdell and Board (1967) showed that the numbers of Gram-negative aerobic bacteria, which were the numerically dominant contaminants at the point of manufacture, diminished within the first 24 hours of storage at 4°C during which time Br. thermosphacta, lactobacilli, and yeast flora were selected. A similar trend was noted with the unsulphited

American fresh pork sausage (Sulzbacher and McClean, 1951; McClean and Sulzbacher, 1953). Further surveys of many well known brands of pork and beef sausages obtained from retail outlets (Dowdell and Board, 1968) revealed that : (1) the microbial flora of both pork and beef sausages were similar in size and composition; (2) there was a wide range in the levels of the initial infection, (pork 1.0×10^5 - 1.32×10^8; beef $5.8\ 10^4$ - $5.0 \times 10^7 g^{-1}$) (3) the level of contamination of individual brands tended to fall within part of the overall range and (4) the scatter of counts for a particular brand increased with an increase in the average level of contamination. These authors contended that the size of the initial microbial population was an important determinant in the selection of the microbial association. Using this criteria they assigned the 48 samples to one or other of the following "microbial groups". Group A - sausages in which there was a low initial microbial load ($5\text{-}10 \times 10^5$ organisms g^{-1}) and in which yeasts became numerically dominant with storage: Group B - sausages which contained a moderate initial infection ($1 - 10 \times 10^6$ organisms g^{-1}) and in which unidentified Gram-positive organisms predominated in the spoilage flora developing with storage [the authors suspected that abnormal storage conditions such as deep freezing of the sausages may have been responsible for this situation]: Group C - sausages in which there was a moderate to high initial infection ($1 - 100 \times 10^6$ organisms g^{-1}) and in which Br. thermosphacta became dominant and Group D - sausages

obtained solely from butchers shops which contained a very high level of contamination (1 - 10 x 10^7 organisms g^{-1}) at the time of manufacture; the flora was dominated by Gram-negative bacteria. These observations led the authors to conclude that the occurrence of yeast (Group A) and Br. thermosphacta (Group C) dominated floras, which were found in 20 and 60% respectively of the total number of sausages surveyed, was a factory specific phenomenon. It is noteworthy that subsequent studies have confirmed these observations without providing an explanation of the underlying causes (Brown, 1977; Abbiss, 1978; Leads, 1979). Subsequently, Dowdell and Board (1971) categorised the microbial contaminants as dominant, major and minor according to their contribution to the initial contamination and their fate on storage at different temperatures (Table 4). The microbial association developing in sausages stored at 4°C was dominated by Br. thermosphacta and yeasts whereas that in sausages stored at room temperature contained lactobacilli and micrococci also. The existence of the microbial association has been confirmed by Brown (1977), Abbiss(1978) and Leads (1979) but not by Ashworth et al (1974). The last mentioned investigated sausages produced on a small scale in an experimental kitchen. Extensive studies at Bath University (Brown, 1977; Abbiss, 1978; Leads, 1979; Banks, 1983) have demonstrated repeatedly that sausages with unusual associations of micro-organisms are produced when materials from a general meat market are used in the small-scale production of sausages. Brown (1977) was probably the first worker to suggest that the two important microbial

Table 4 Classification of contaminants comprising the microbial association of the British fresh sausage at the time of manufacture and after storage.

Group	Contaminant	Initial inoculation (c.f.u. g^{-1})	Growth at 4 (°C)	Growth at 22 (°C)
Dominant	*Microbacterium thermosphactum*	10^5	+	+
	Micrococcus spp.	10^4	-	+
	Lactobacillus spp.	10^2 - 10^4	+	+
	Yeasts	10^3 - 10^5	+	+
Major	*Streptococcus faecalis*	10^2 - 10^4	w	w
	Streptococcus faecium			
	"Coliforms"	10^2	w	w
Minor	*Kurthia zopfii*	$<10^5$*	-	-
	Pediococcus spp.	$<10^2$	-	-
	Leuconostoc spp.	$<10^2$	-	-
	Bacillus spp.	$<10^2$	-	-
	Clostridium spp.	$<10^2$	-	-
	Pseudomonas spp.	10^4 - 10^5	-	-
	Acinetobacter spp.			

Adapted from Dowdell & Board (1971)

* Recovered from only 4 samples
\+ Recovered at this concentration in heavily contaminated samples only
w weak growth

associations (A and B above) developed as a consequence of sulphite selectiom. The selective role of sulphite was confirmed by Banks and Board (1981) (see pp 14-17 also) whose ranking of the sulphite tolerance of yeasts and bacteria isolated from sausage - Yeasts > Br. thermosphacta, lactobacilli, streptococci > pseudomonads > enterobacteria least tolerant - was mirrored in the extent of growth of these organisms in sausages stored at 4 10, 15 and 25°C. It needs to be stressed that, as sulphite binding occurs during the storage of sausages, some organisms such as pseudomonads remain quiescent until the concentration of free sulphite falls below an inhibitory level.

The effect of temperature

It is generally accepted that the temperature of storage drastically influences the type and extent of spoilage of meats (Ingram and Dainty, 1971). As mentioned previously (pp 31-35) the composition of the microbial association of sausage is influenced by the temperature of storage (Dowdell and Board, 1971). Storage at 4°C, for example, leads to the establishment of a Br. thermosphacta and a yeast dominated flora, whereas at 22°C the occurrence of large populations of lactobacilli and micrococci were also evident. The rate of growth of the microbial association is considered to be influenced by storage temperature such that the time taken to achieve climax populations increases with decreasing temperature - from 3 to 7 d at 22 and 4°C respectively. (Abbiss, 1978). The onset of spoilage, which has been shown by

the taste panel surveys of Leads (1979), to be undetectable by the consumer until after climax populations have been obtained, will therefore be delayed. Dyett and Shelley(1962, 1966) were perhaps the first to note that the efficacy of sulphite was enhanced by refrigerated ($4^{o}C$) storage. Indeed, the addition of sulphite to vacuum packed bacon burgers resulted in progressive extension of the shelf life as the storage temperatures were reduced (e.g. 2, 5, 10 d at 22, 10 and $4^{o}C$ respectively, Gardner, 1968). Banks (1983) surmised that temperature exerted an important effect on the efficacy of sulphite as a preservative in the British fresh sausage. This author noted that concentrations of bound sulphite increased and those of the free sulphite diminished more rapidly at higher temperatures. Moreover, the preservative appeared to be more inhibitory to lactobacilli, micrococci and pseudomonads at storage temperatures below $10^{o}C$. As noted above (pp 15-16) the time of storage appears to influence the growth of some microbial contaminants. The growth of pseudomonads, for example, has been shown to occur towards the end of storage (Ashworth *et al.*, 1974; Banks, 1983) at 22 and $4^{o}C$ when the concentrations of free sulphite have fallen below a critical level (110 μg^{-1}; Banks, 1983).

The effect of site

Dowdell and Board (1971) found that populations of the microbial contaminants, other than lactobacilli, at the surface were larger than those at the core of sausages. The populations

of lactobacilli from both sites were of similar size. Subsequent investigations (Brown, 1977; Abbiss, 1978 and Leads, 1979) have confirmed the former but not the latter observations. It is generally accepted that the extrinsic properties of the surface and core of meat differ (Mossel, 1971). The concentration of oxygen, for example, rapidly diminish in the core of meat as a consequence of oxidative reactions (Lawrie, 1974). The rate and extent of growth of organisms in meat is considered to be proportional to oxygen availability (Mossel, 1971) and, therefore, growth of micro-organisms in the core location will be limited by the rate of diffusion of oxygen from the surface. Abbiss (1978) was of the opinion that a similar situation obtained in sausages and also that in a package of 6 - 8 sausages the oxygen availability was highest at the outer surfaces of sausages, especially those in contact with the oxygen permeable cellophane wrapper. This author could not demonstrate, however, any appreciable differences between the microbial populations recovered from the surface of sausages located at the centre and edge of the pack suggesting that differences in oxygen availability at the surface were minimal.

The effect of heat treatment

The British fresh skinless sausage, which as previously mentioned (p 5) differs from the standard fresh sausage in that the the proteins at the periphery are coagulated by heat, have been shown to support the growth of Br. thermosphacta, yeasts,

lactobacilli and pseudomonads (Hockley, 1980; Legan, 1981; Fielder, 1983). The heat treatment during processing has a pasteurising effect on the micro-organisms causing a 10 - 100 fold reduction in the level of infection at the surface of skinless sausages (Hockley, 1980; Legan, 1981; Fielder, 1983). Moreover, Fielder (1983) observed that a resuscitation stage in the recovery of heat stressed micro-organisms increased the recovery of organisms from the surface of such sausages. This worker as well as Hockley (1980) and Legan (1981) noted that the rate and extent of growth of micro-organisms other than lactobacilli and streptococci during storage at temperatures of 25°C or less were greater at the surface than the core. A similar situation obtains at the surface of the British fresh sausage (Dowdell and Board, 1971; Brown, 1977; Abbiss,1978; Leads, 1979; pp 36-37) which has attributed to greater oxygen availability at the surface location (Abbiss, 1978). Legan (1981) was of the opinion that, in skinless sausages, this situation may be due to the release of nutrients at the periphery by thermal denaturation of proteins. He noted also the development of a yeast-dominated flora at the surface of skinless sausages during storage at refrigerated temperatures (4°C). It is well known that the microbial association which develops in standard fresh sausage with a low initial level of contamination is usually yeast dominated. Dowdell and Board (1971) and Fielder (1983) suggested, therefore, that this situation arose from heat treatment reducing the level of bacterial but not yeast

contamination. The climax populations of microbial contaminants of the skinless sausage have been shown to be larger than those in British fresh sausage stored under similar conditions (Hockley, 1980; Legan, 1981; Fielder, 1983). This may be associated with the rapid loss of free sulphite from the surface of skinless sausages, such that minimal concentrations of active free sulphite exist when climax populations are achieved (Legan, 1981). Indeed, it is generally accepted that the higher climax populations are attained in British fresh sausages in the absence of preservative (Dyett and Shelley, 1962 and 1966; Brown, 1977). The concentration of free and bound sulphite at the surface but not the core of skinless sausage has been shown to decrease markedly during heat treatment as a consequence, it was assumed, of the diffussion of sulphite into the heating water (Legan, 1981). Fielder (1983) recovered only small quantities of sulphite (ca 2.5 mg l^{-1}) in the heating water; he surmised that haem-catalysed breakdown (Brown, 1977) of sulphite was probably involved. The concentration of free sulphite is reported to decrease yet further in stored skinless sausages, this effect being greater at the surface than at the core site (Legan, 1981; Fielder, 1983). This loss and also the extent of binding was greater at high storage temperatures. Indeed, these observations are in agreement with those for British fresh sausages (Banks, 1983).

Yeasts in meat products

The source and activities of Br. thermosphacta lactobacilli, pseudomonads and enterobacteria in meat and meat products is well documented. For the purpose of this study, only the literature pertaining to the source, identity and activity of yeasts in the meat industry will be reviewed in detail.

It is evident from the limited literature that yeasts occur throughout the meat industry (Walker and Ayres, 1970). Ingram, (1958) considered that the ability of a yeast to compete with other organisms and contribute to spoilage in foods was influenced by the pH, redox potential (Eh), water activity (aw) and nutrient availability. The concentration and composition of the initial level of contamination and their rate of growth at refrigeration temperatures is lower than the predominant bacterial contaminants (Ayres, 1960). It is generally accepted that yeasts contribute to the spoilage of meat only if the growth of the bacteria is arrested (Walker and Ayres, 1970) as has been shown to occur on chilled and eviscerated chlorotetracyline-treated poultry (Njoku-Obi, et al.,1957; Wells and Stadelman, 1958; Walker and Ayres, 1959). Yeasts are ubiquitous in nature (Lodder, 1970) and are considered to be introduced into a meat processing factory via the hair, hides and viscera of animals (Emprey and Scott, 1939; Ayres, 1955). Lea (1931a) attributed the increase in the level of contamination of mutton carcasses stored at -5°C to the growth of unidentified yeasts.

On extended storage discrete yeast colonies developed on the internal flanks but these were not considered to contribute to spoilage. The growth of unidentified yeasts on chilled beef and moist muscle was considered to be the major cause of beef fat lipolysis (Lea, 1931b) and the production of off odours (Scott, 1936). In fact, the yeasts were not considered to be the numerically dominant microbial contaminant of the former. Ingram (1958) surmised that microbiologists underestimated the role of lipolytic yeasts in meat spoilage. Yeasts belonging to the asposporogenous genera Candida, Torulopsis and Rhodotorula have been isolated from choice beef stored at refrigerated temperatures (Ayres, 1960). Low numbers of Candida, Debaryomyces, Rhodotorula and Torulopsis have been recovered from cured meats such as hams (Ingram, 1952) and bacon (Gardner, 1971) but, yet again, their contribution to spoilage was deemed to be negligible. Recently, Lowry and Gill (1984) demonstrated the development of a yeast-dominated flora on frozen lamb stored at $-5^{\circ}C$ for up to 40 weeks. They identified their isolates with Cryptococcus laurentii var. laurentii, Cr. infirmominatus, Trichosporon pullulans and Candida zeylanoides. The first mentioned species was reported to form over 90% of the yeast flora at all times. Comminution of joints of meats leads to yeasts being dispersed throughout the minced meat; the number of such organisms tends to be relatively low ranging from 2×10^{1} to 6×10^{4} organisms g^{-1} (Jay and Margitic, 1981). The identity of these yeasts and their role, if any, in spoilage has

been ignored. The earliest reports of yeast spoilage in sausage-type meats were those of Kuhl (1910), who recovered a white colony forming, non-fermentative yeast from the surface slime of dried sausages, and Mrak and Bonar (1938) who identified yeast cultures which had produced surface slime in Weiner sausages with Debaryomyces guillermondii var. nova Zeelandicus (subsequently re-identified with D. hansenii Lodder, 1970). Drake et al. (1958, 1959) isolated this species and occasionally Candida, Torulopsis and Trichosporon spp. from the surface slimes of frankfurters. Even though their numbers were smaller than those of bacteria the growth of unidentified yeasts in British fresh sausage during storage at 22°C has been associated with the production of off odours (Dyett and Shelley, 1966). Dowdell and Board (1971) concluded, from an extensive survey of British fresh sausages, that yeasts, although not a numerically dominant component (1×10^1 - 2.4×10^4 g^{-1}) of the microbial association, ought to be regarded as a major component of the microbial association in terms of biomass. These authors did not identify any of their isolates but they did demonstrate that the majority were non-fermentative. Abbiss (1978) confirmed these findings and identified 50 isolates obtained from sausage with C. lipolytica, C. parapsilopsis, C. zeylanoides, Cr. laurentii, Rhodotorula glutinis, Rh. rubra Torulopsis candida, Tr. cutaneum and Tr. pullans. The growth of unidentified yeasts in sausage has been reported by many workers, notably Dowdell and Board (1968, 1971),

Ashworth *et al.* (1974), Brown (1977), Banks and Board (1982) and Banks (1983), but not Dyett and Shelley (1966), to be unaffected by the legally permitted levels of sulphite. The last mentioned reported that sulphite retarded yeast growth such that climax populations in sulphited sausages stored at 22°C were 1 - 2 log cycles lower than those in unsulphited ones. In the majority of studies of the microbial associations, the yeasts appeared to have the greatest tolerance of the preservative (Brown, 1977; Banks, 1983) and Brown (1977) concluded that they were a source of, as yet unidentified, compounds which, through the binding of sulphite, progressively diminished the active concentration of the preservative. It is possible to conclude from the limited literature that little attention has been given to the identity of the yeasts recovered in the majority of studies.

Previous work on the microbiology of sausage has been concerned primarily with the identification of the bacterial components of the microbial association. Until the identity of the yeasts in sausages has been established, little headway will be possible in the studies of the production and identity of yeast-mediated sulphite-binding compounds or of the contribution of these organisms to spoilage. For this reason the present study was undertaken with the objective of identifying the principal yeast contaminants from sulphited and unsulphited sausages and also minced beef and to establish whether or not sulphite elected a yeast flora of particular composition.

Yeasts from skinless sausages were studied with the objective of establishing whether or not the heat processing had an elective action such that ascosporogenous ones were favoured as is the case with other heat treated foods and beverages (Put and De Jong, 1980). A large sausage factory with its own abattoir was screened for the presence of yeast in order to determine the source of yeast infection.

METHODS AND MATERIALS

METHODS AND MATERIALS

Source of sausage samples

Batches of sausages wrapped in food grade polyethylene films (oxygen and water vapour permeability 400 cm^{-2} h^{-1} 0.025 mm^{-1} and 5g m^{-2} $24h^{-1}$ 0.025 mm^{-1} respectively at 25°C and, in the case of the latter, 75% relative humidity) in 1 lb packs of 8 were obtained directly from a large local factory. All except one batch contained legally permitted concentrations of the preservative (sodium metabisulphite), which was included in the seasoning (Rank Hovis MacDougall 599, England) at a predicted final concentration of ca 600 µg SO_2 g^{-1} sausage.

In addition 1 lb packs of pork sausages from retail outlets were examined also. In practice, the storage histories of the packs before sampling were unknown.

Storage of sausage

In keeping with factory practices sausages were stored with access to air either at 4°C for 24 hours and thereafter at 1, 4 or 15°C for up to 10 days.

Preparation of sulphited pork belly mince

(1) Sulphite solutions

As it is generally accepted that the concentration of sodium metabisulphite in a filter sterilised solution is reduced as a consequence of sulphite binding to the cellulose acetate membrane

(Banks, 1983; Stratford, 1983), sulphite solutions were prepared by the direct addition of sodium metabisulphite to sterile distilled water. Preliminary investigations revealed that such solutions, when inoculated into Erlenmeyer flasks (250 ml) containing sterile culture media (150 ml tryptone soya broth (lab m) or lab lemco broth Oxoid), did not cause contamination.

(2) Seeding of pork belly mince with sulphite

Pork belly mince, obtained directly from a local factory, was weighed into a clean plastic bowl in 1 kg quantities, to which was added, during thorough mixing by hand (wearing an alcohol 70%, cleaned glove (Traveanol), 50 ml of a freshly prepared sulphite solution (0.5 $g\,l^{-1}$), which was predicted to give a final concentration of 500 $\mu g\,g^{-1}$ minced belly pork. Control samples, to which 50 ml of sterile distilled water had been added, were prepared also. Samples (150g) were put into plastic trays (9 x 18 cm, Thorpac Limited, Norway) and were covered and heat sealed with a transparent film (cellophane, British Cellophane).

(3) Seeding of sulphited pork belly mince with glucose

Weighed quantities of freshly prepared sulphited pork belly mince (as described above) were thoroughly mixed (as described above) in a clean plastic bowl with 50ml of a glucose solution,

which had been prepared as described for sulphite solutions, such that a final concentration of 20 mg g^{-1} minced pork was achieved. Control samples were prepared and the samples were packed as previously described.

(4) Seeding of sulphited pork belly mince with micro-organisms

Members of the microbial association of sausage namely : a yeast (D. hansenii); Br.thermosphacta; a Lactobacillus and a pseudomonad, which had been isolated from sausage were inoculated into Erlenmeyer flasks (250 ml) containing sterile tryptone soya broth (pH 7, lab m) and were incubated at 25°C for 12 h. The organisms were then harvested aseptically by centrifugation (4,500 g, 15 min), resuspended in sterile distilled water (50 ml) and inoculated at a level calculated to give a final concentration of ca 1 x 10^5 organisms g^{-1} of minced pork into weighed quantities (1 kg) of freshly prepared pork belly mince to which sulphite (500 µg g^{-1}) and glucose (20 mg g^{-1}) had been added. Samples containing final concentrations of the yeast, D. hansenii, of ca 1 x 10^6 and 1 x 10^7 and control samples to which sterile distilled water had been added (50 ml) were prepared also. The inoculated meat slurries were mixed thoroughly by hand and samples were packaged as previously described.

Storage and sampling

Samples were taken immediately after preparation and daily, thereafter, during storage at 1° and 15°C.

Chemical analyses

Determination of sulphite concentration

The concentrations of free, bound and total sulphite were determined by the method of Banks and Board (1982a).

Sample preparation

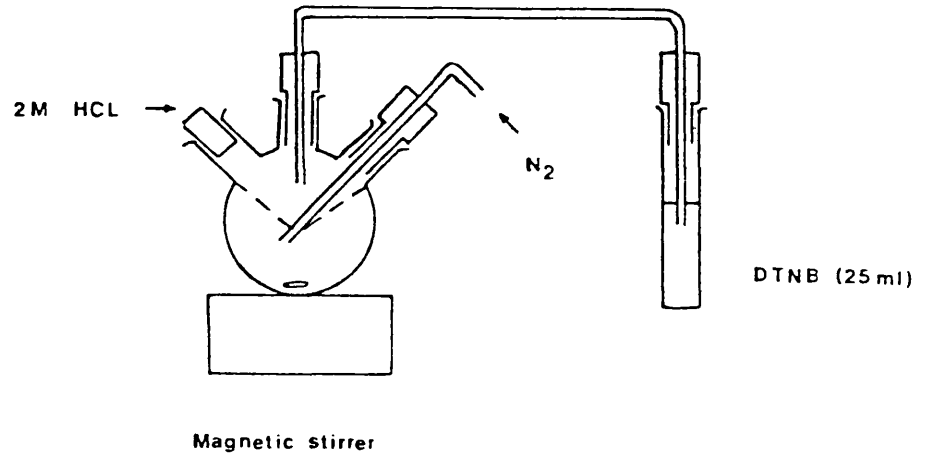
A sample (5g) of sausage or minced pork was added to chilled deoxygenated distilled water (20 ml) in a screw cap bottle (50 ml) containing glass beads and shaken 60 times through an arc of 0.6 m for 30 seconds.

Free sulphite determination

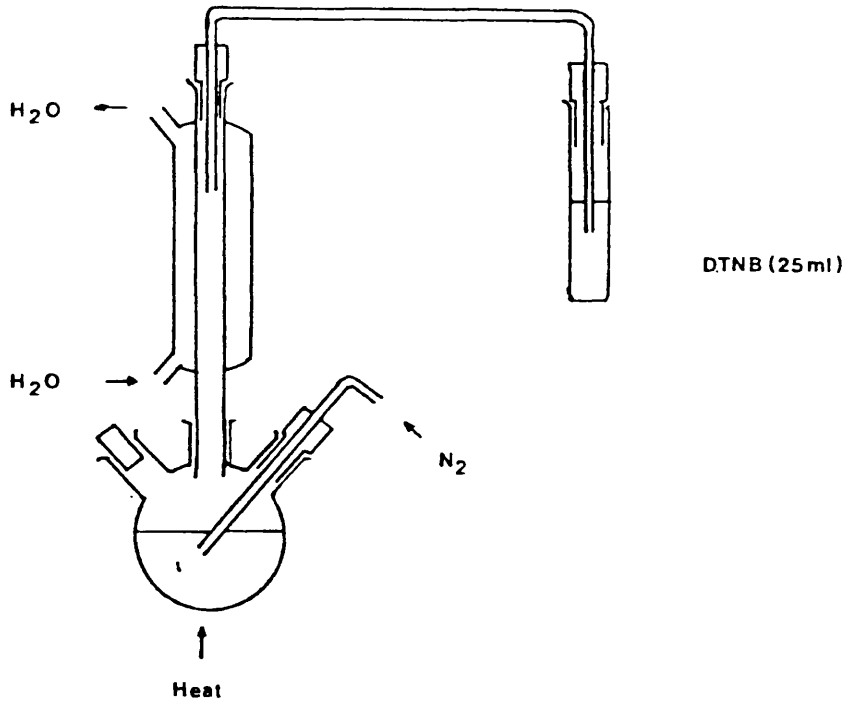
The specimen was transferred immediately to a three necked round bottomed flask (Q and Q FR 250/35/22A) containing a magnetic stirring bar (Figure 4a). One side neck was connected to a supply of nitrogen (Air Products, Bristol) *via* a modified Dreschel bottle head (Q and Q 27/3/13). A Dreschel bottle head was connected to the centre neck and also a second Dreschel bottle head, the dip tube of which was placed in a boiling tube (Q and Q MF 24/3) below the surface of a 2.3×10^{-3} M solution of 5-5- dithiobis 2 - nitrobenzoic acid (25 ml, DTNB) dissolved in a 2 M phosphate buffer (pH 8) containing ethanol (10% w/v). Nitrogen was passed through the system (20 $cm^3 . 5.^{-1}$, Air Products, Bristol) and hydrochloric acid added *via* the remaining side neck (20 ml). After 15 minutes incubation the DTNB was added to phosphate buffer (25 ml) and the absorbance measured at

Figure 4 Diagrammatic representation of the apparatus used for the determination of (A) free and (B) bound sulphite.*

A Free



B Bound



* Adapted from Banks and Board (1982a)

412 nm (Pye Unicam SP 6 550 UV/VIS). The absorbance was related to sulphite concentration by a standard curve obtained from iodometrically standardised sulphite solutions.

Bound sulphite determination

The distillation flask (above) was transferred to a heater bed and a vertical condenser was connected to the centre neck to which two Dreschel bottle heads attached in series (as described above) were placed (Figure 4b). Nitrogen was passed through the system and the flask was heated for 15 minutes. The analysis of DTNB was done as for free sulphite.

Total sulphite determination

The concentration of total sulphite was determined by the summation of values of free and bound sulphite.

pH determination

The pH of the aqueous layer of a sausage homogenate (p 51) was determined with a glass electrode (Russel, England).

Glucose concentration

The samples were prepared and the concentration of glucose was determined using the Boehringer Mannheim test combination kit for glucose (Cat. 124010).

Microbial analyses

Sampling

A sterile scalpel was used to cut longitudinally the skins (casings) of three sausages from 8 or so in a polyethelene wrapped pack. A sterile spatula was used to scoop out a composite sample of 20 g which was homogenised for 60 seconds in 180 ml of quarter-strength Ringers solution with a Colworth Stomacher 400 (Seward, London). A composite sample of minced pork was treated similarly.

Isolation, enumeration and maintenance of micro-organisms

Yeasts

Samples (0.1 ml) of an appropriate dilution of the homogenate in quarter-strength Ringers solution were spread over the dried surface (37°C for 1.5 h) of Rose bengal chloramphenicol agar (RBG, lab m). Three replicates were done at each dilution level and incubation was at 15°C for 5 days in the dark. This medium and incubation regime were adopted after an initial survey in which the numbers of colonies developing on acidified (pH 3.5) malt extract agar (ME*, Holwerda, 1952), acidified (pH 3.5) plate count agar (PCA*, Dowdell and Board, 1968), oxytetracycline glucose yeast extract agar (OX1, Mossel et al., 1970) and Rose bengal chloramphenicol agar (lab m) incubated at 4, 15 and 25°C, were compared daily for up to 7 days (Tables 5 and 6). Although malt extract gave a slightly

Table 5 The mean colony counts g^{-1} of sausage recovered by 4 yeast-selective media.

Incubation Temperature (°C)	Acidified (pH 3.5) malt extract (ME*)	Acidified (pH 3.5) plate count agar (PCA)	Oxytetracycline glucose yeast extract agar (OXI)	Rose-bengal chloramphenicol agar (RBC)
25	181.9×10^5	117.5×10^5	108.3×10^5	104.6×10^5
	(2) M	(2) M	(2) M	(5) m
15	186.7×10^5	135.0×10^5	135.0×10^5	144.8×10^5
	(3) M	(3) M	(3) M	(7) m
4	185.5×10^5	125.0×10^5	153.0×10^5	63.8×10^5
	(7) M	(7) M	(7) M	(10) m

Preparation of Plates:- 0.1 ml amounts of appropriate sausage dilutions were inoculated onto 5 replicates of each media and incubation regimes

Code:- M extensive mould growth

m restricted mould growth

Incubation time (d) in parenthesis

Table 6 The significance of the differences of yeast colony counts g^{-1} sausage by 4 yeast selective media.

$RBC^{(25)}$	$RBC^{(15)}$	$RBC^{(4)}$	$ME^{(25)}$	$ME^{(15)}$	$ME^{(4)}$	$OXI^{(25)}$	$OXI^{(15)}$	$OXI^{(4)}$	$PCA^{(25)}$	$PCA^{(15)}$	$PCA^{(4)}$	
-	S	S	S	S	S	NS	NS	S	NS	NS	NS	$RBC^{(25)}$
	-	S	S	S	S	S	S	NS	NS	NS	S	$RBC^{(15)}$
		-	S	S	S	S	S	S	S	S	S	$RBC^{(4)}$
			-	NS	NS	S	S	S	S	S	S	$ME^{(25)}$
				-	NS	S	S	S	S	S	S	$ME^{(15)}$
					-	S	S	S	S	S	S	$ME^{(4)}$
						-	NS	S	NS	S	S	$OXI^{(25)}$
							-	NS	NS	NS	NS	$OXI^{(15)}$
								-	NS	NS	NS	$OXI^{(4)}$
									-	NS	NS	$PCA^{(25)}$
										-	NS	$PCA^{(15)}$
											-	$PCA^{(4)}$

continued

Table 6 continued

Code :-

* - significance calculated by using the student 't' test

S - significant difference i.e. $p < 0.05$

NS - not a significant difference i.e. $p > 0.05$

RBC - Rose-bengal chloramphenical agar

ME - Acidified (pH 3.5) malt extract agar

OXI - Oxytetracyline glucose yeast extract agar

PCA - Acidified (pH 2.5) plate count agar

Incubation temperature (^{o}C) in parenthesis

larger count the unrestricted growth of moulds made isolation of yeasts difficult. Colonies were selected from the plates incubated with the highest dilution and subcultured on Sabouraud dextrose agar (Oxoid). Incubation was for 3 days and isolate purity was checked microscopically. Isolates were stored on slopes of Sabouraud dextrose agar at 4°C and subcultured every 8 weeks.

Brochothrix thermosphacta

A sample (0.1 ml) of an appropriate dilution was spread over the dried surface (37°C, 1.5 h) of streptomycin thallous acetate actidione agar (STAA, Gardner, 1966). Three replicates were done at each dilution level and incubation was at 20°C for 3 days. The selectivity of the medium was checked regularly by microscopic examination of Gram-stained smears of colonies picked at random.

Lactobacilli

A sample (1 ml) of an appropriate dilution was inoculated by the pour plate method into 10 ml of the lactobacilli selective medium of (Keddie, 1951). When set, an overlay (10 ml) of the medium was added. Three replicates were done at each dilution level and incubation was at 25°C for 5 days. Medium selectivity was checked regularly by microscopic examination of Gram-stained smears of colonies selected as described for the yeasts.

Pseudomonads

Samples (0.1 ml) of an appropriate dilution were spread over the dried (37°C for 1.5 h) surface of Cetrimide-fusidic acid cephalodrine medium (CFC, Mead and Adams, 1977). Incubation was at 15°C for 48 h and 3 replicates of each dilution were done. Medium selectivity was checked by regular microscopic examination of Gram-stained smears of colonies selected as described for the yeasts.

Enterobacteria

A sample (1 ml) of an appropriate dilution was inoculated by the pour plate technique into 10 ml of molten (45°C) Violet red bile glucose agar (VRBG, OXOID). When set, the agar was overlayed with VRBG (10 ml). Three replicates were done at each dilution level and incubation was at 37°C for 24 h. Medium selectivity was checked by regular microscopic examination of Gram-stained smears of colonies selected as described for the yeasts.

Total viable count

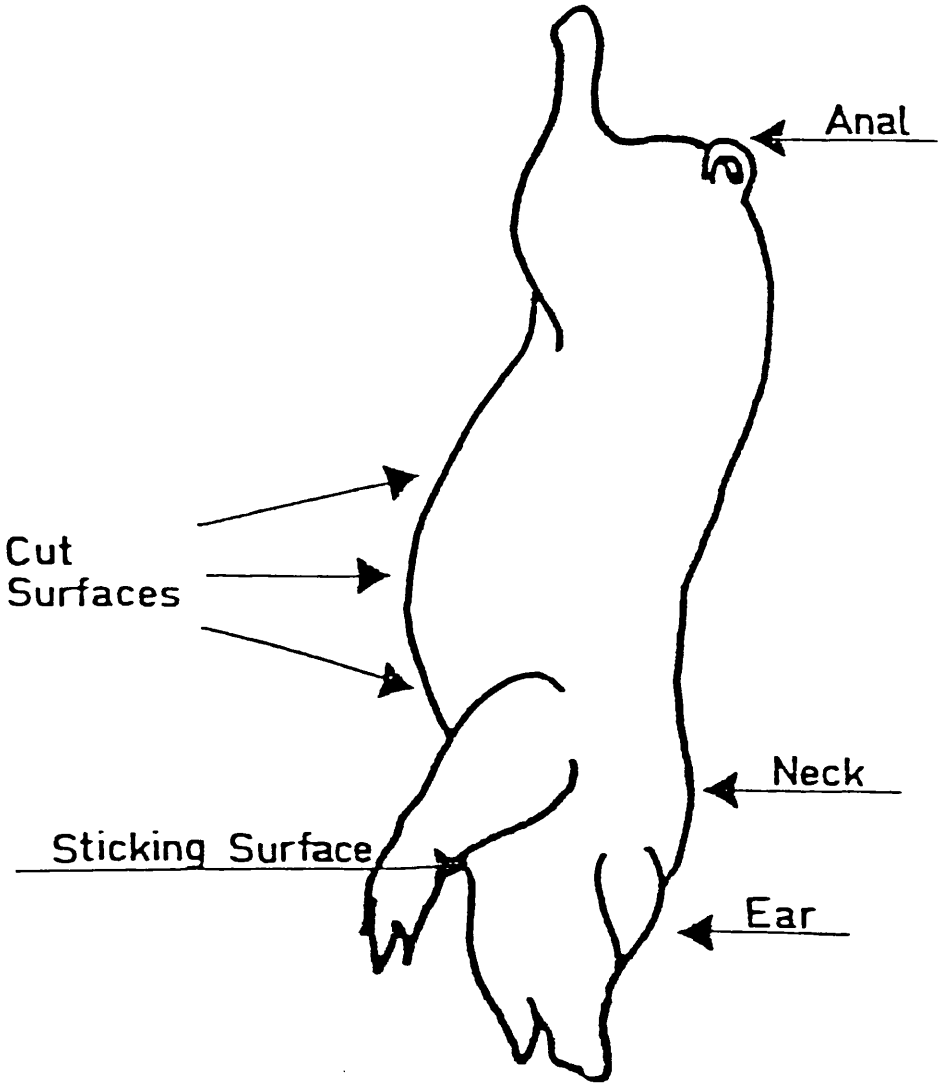
A sample (0.1 ml) of an appropriate dilution was inoculated onto the dried surface (37°C,1.5 h) of plate count agar (PCA lab m). Incubation was at 25°C for 4 days and three replicates of each dilution were done.

Factory survey

Sampling

The pig carcasses, which were examined in the study of the route of yeast infection in the factory, were selected at random from those being sampled during a normal day's kill. In practice, sampling had to be completed within 18 seconds at one stage. In consequence, the swab method of sampling was chosen for the purpose of speed even though the variability of this method is well known. In practice, this feature was of minor concern because attention was being directed at types rather than numbers of yeasts in the phase of this study. Surface swabs were taken from behind the ear, the neck, the anal region, the sticking surface, the swabbing being done immediately after slaughter, after washing and evisceration and after 24 hour storage at $4^{o}C$ (Figure 5). Swab samples of cut surfaces were taken also after evisceration and after 24 hours chilled storage at $5^{o}C$. Swab samples of the cut surfaces were taken also after evisceration and chilled storage. Additional swab samples were taken from the lairage walls and floors and equipment in general use in the factory. Sterile metal guides having an exposed inner area of 10 cm^2 were used to mark the sampling position. Each area was then swabbed with a sterile cotton tipped applicator, moistened with sterile quarter-strength Ringers solution. Dilutions were prepared as previously described. In addition 20 g samples of head and belly meat,

Figure 5 Diagram to show the pig carcass sampling sites.



back fat and rind from butchered carcasses and all the constituents used in the final sausage mix were sampled, as previously described for sausage and minced pork samples. Yeasts were isolated and enumerated as previously described (p 51).

Aerial yeast contamination was measured using a Biotest RCS Air sampler (Birmingham, England) in the following areas : (1) the slaughter line; (2) the dehairing areas; (3) the gutting and evisceration areas; (4) the cold storage room; (5) the butchery department; (6) the chopping room, and (7) the sausage production line area.

Yeast identification

As far as possible all yeast isolates were classified according to the taxonomy proposed by Lodder (1970) after characterisation by the following methods :

Macromorphology

The gross morphology, pigmentation and texture of a streak culture on malt extract (6%) agar after incubation at 25°C for 3 weeks was noted. The medium contained (g l^{-1}) malt extract (6%), agar No. 2 (1.5%, lab m) and distilled water to 1 l.

Micromorphology

The characteristics of vegetative cells taken from cultures

on slopes of malt extract (6%) agar, after incubation at 25°C for 3 days, was observed microscopically. Length and breadth measurements were done using an image splitting eye piece. The production of pseudomycelium, true mycelium, ascospores, arthrospores and ballistospores was determined by direct microscopic examination of streak cultures, partially covered by a sterile coverslip on corn meal agar (Oxoid) after 4 days incubation at 25°C.

Fermentation

The fermentation tests were done with a modification of the method of Beech et al. (1968). Test tubes containing a 25 x 5 mm Durham tube, 0.5 ml of filter sterilised solution of 6.7% (w/v) yeast nitrogen base (Difco) and 2% (w/v) of the carbon source under test and 4.5 ml of sterile distilled water were inoculated with 0.1 ml of a yeast suspension prepared from actively growing malt extract cultures as described by Wickerham (1951). The test tubes were incubated at 25°C and observed regularly for 4 weeks for gas production. All yeast isolates were screened for their ability to ferment glucose; only glucose fermenting isolates were subjected to further fermentation tests incorporating cellobiose, galactose glycerol, lactose, maltose, melezitose, melibiose, mannitol, raffinose, soluble starch and trehalose. Raffinose was used at a concentration of 6% (w/v).

Production of urease

Urea hydrolysis was detected by the emergence of a pink colouration around a yeast colony on urea agar (Difco) after incubation at 25°C for 24 h. The medium was prepared according to the manufacturer's instructions and dispensed (25 ml) into 25 well Petri dishes (2 x 2 cm, Sigma). Washed standardised suspensions were prepared and inoculated as for the fermentation tests.

Assimilation of carbon compounds

The carbon assimilation tests were done with a modification of the method of Beech et al. (1968); one part of a filter sterilised solution of 6.7% (w/v) yeast nitrogen base (Difco) and 2% (w/v) of the carbon source to be tested were added to 9 parts of sterile 1.1% (w/v) purified agar (Oxoid) at 45°C. The medium was poured (20 ml) immediately into Petri dishes and dried (37°C for 1.5 h). Washed and standardised inocula (Wickerham, 1951) were prepared from yeast cultures which had been incubated (25°C for 4 d) on a starvation medium, the test medium containing only 0.1% glucose. The Petri dishes, which were inoculated with 21 isolates by a Denly Multipoint inoculator (Surrey), were incubated at 25°C and examined after 3, 5 and 7 days. The carbon compounds tested included : adonitol; D-arabinose; L-arabinose; arbutin; cellobiose; citric acid; erythritol; ethanol; galacticol; glucosamine hydrochloride;

galactose; glucitol; gluconic acid; glycerol; inositol; inulin; lactic acid; lactose; maltose; maltotriose; mannitol; mannose; melibiose; melezitose; ∝ methyl D-glucopyranoside; raffinose; ribitol; ribose; salicin; sorbitol; sorbose; starch; succinic acid; sucrose; trehalose; xylitol; xylose. Positive and negative control media containing glucose and no carbon source were prepared also and control Petri dishes were inoculated at the beginning and the end of the inoculation series.

Assimilation of nitrate

An agar modification of the nitrate assimilation medium of Beech et al. (1968) was used; 1 part of a filter sterilised solution of 11.7% (w/v) yeast carbon base (Difco) and 0.78% (w/v) potassium nitrate (BDH) wereadded to 9 parts of a sterile 1.1% (w/v) purified agar (Oxoid). The medium was poured (20 ml) into Petri dishes and dried (60°C for 1.5 h). A control medium lacking potassium nitrate was also prepared. Cell suspensions were prepared and inoculated as for the carbon assimilation tests except they were starved on the control nitrate medium (25°C for 4 d).

Hydrolysis of fat

The lipolytic activity of all isolates was judged by their action on Tweens (20, 40, 60 and 80) using Sierra's (1957)

Tween medium. Washed and standardised cell suspensions were prepared and inoculated as described for the carbon assimilation tests.

Gelatin hydrolysis

The proteolytic activity of all isolates was judged by their ability to liquify gelatin using Wickerham's (1951) synthetic gelatin medium. This medium was poured (3 ml) into 25 - well Petri dishes (2 cm x 2 cm, Sigma) and cooled (15°C). Washed and standardised cell suspensions were prepared and inoculated as for the fermentation tests. Incubation was at 15°C for 4 weeks.

Growth at 5 and 37°C

The extent of growth of yeast isolates on malt extract (2%) agar incubated at either 37 or 5°C for 5 and 10 days respectively was noted. Cell suspensions were prepared and inoculated as for the carbon assimilation tests.

Bismuth sulphite tolerance

Yeast cell suspensions were prepared and inoculated as previously described for carbon assimilation tests, into Petri dishes containing mixtures of bismuth sulphite agar (lab m) and malt extract (3%) agar, in which the concentration of bismuth sulphite agar was 20% 50% and 80%.

Stock cultures

Numbered (in parenthesis) stock cultures obtained from the National Yeast Culture Collection (Norwich, England) of the following species :- Candida humicola (461); C. lipolytica var. deformans (376); C. lipolytica var. lipolytica (789); C. mesenterica (390); C. valida (327); C. vini (331); C. zeylanoides (462); Cryptococcus albidus var. aerius (445); Cr. laurentii var. laurentii (139); Cr. marcerans (571); Debaryomyces hansenii (793); Pichia membranaefaciens (169); P. vini var. vini (741); Rhodotorula glutinis (59); Rh.rubra (64); Torulopsis candida (611) were used to check the characterisation methods used in this study.

Determination of extracellular amylase, lipase and protease activity in pure culture

Amylase

Yeast isolates were inoculated into Erlenmeyer flasks (250 ml) containing a sterile (121°C for 15 minutes) starch medium (150 ml, pH 6.8, Abbiss, 1978), and were incubated in a shaking water bath (90 oscillations min^{-1}) at 25 and 4°C for 24 h and 6 d respectively. The test medium contained (g l^{-1}) mycological peptone (lab m, 20) yeast extract (lab m, 0.6); dipotassium phosphate (BDH, 0.4). Samples (1 ml) of the cultures were inoculated into fresh medium and incubated as above. Growth was monitored by absorbance (600nm, Pye Unicam SP6 550 UV/VIS and

related to cell number by a standard curve. Samples (10 ml) were withdrawn from cultures in stationary phase and were centrifuged (4,500g for 15 minutes) and filter sterilised. The filtrate was then assayed for general amylase and glucoamylase activity.

ASSAY

(1) Amylase activity. The ability of a culture filtrate to generate reducing sugars from starch was determined by the modified (Abbiss, 1978) method of Dygert et al. (1975). After an equilibration period (ca 5 minutes) at room temperature, samples (1 ml) were added to a modified sodium borohydride starch solution (1 ml, Strumeyer, 1967) and incubated at 30°C for 60 minutes. The reaction was curtailed by the addition of anhydrous sodium carbonate (4% w/v) glycine (1.6% w/v) copper sulphate (0.045% w/v) reagent (4 ml) and a neucoproine hydrochloride (0.12% w/v) solution (4 ml). The mixtures were incubated in a boiling water bath for 12 minutes and the absorbance was measured (450 nm, Pye Unicam SP6 550 UV/VIS) and related to reducing sugar concentration by a standard curve obtained with standard glucose solutions.

(2) Glucoamylase activity The ability of the culture filtrate to generate glucose from starch was determined by the method of Abbiss (1978). Samples (1 ml) which

had been equilibrated at the reaction temperature (ca 5 min) were added to a 1% (w/v) starch solution in a citrate phosphate buffer (20 mM, pH8) and incubated at 30°C for 120 minutes. The concentration of glucose was then determined using a Boehringer Mannheim test combination for glucose (cat. 124 010).

Lipase

Yeast isolates were inoculated and incubated as described for amylase detection in a sterile (121°C for 15 min) test medium (pH6.8) containing (gl^{-1}): glucose (BDH, 20) urea (BDH, 2); potassium phosphate (BDH, 6); di potassium phosphate (BDH, 2); potassium chloride (BDH, 1); di potassium phosphate (BDH, 2); potassium chloride (BDH, 1); Magnesium sulphate - 7.H_2O (BDH, 0.5); Ferric chloride .6 H_2O (0.01); yeast extract (lab m, 1), olive oil (Sigma, 10). Samples were obtained and prepared as previously described.

ASSAY

The ability of a culture filtrate to release free fatty acids from lipids was determined by the method developed at Bath University (Banks unpublished). Samples (1 ml) were pipetted into screw capped Universals containing (ml): 0.2 M phosphate buffer (2); 0.3 M calcium chloride solution (1); deionised water (5); 2% (w/v) polyvinyl alcohol (1) and the lipid under test (1). The lipids analysed in this study were olive oil,

triacetion, tributyrin, trihexanoin, triolein (Sigma), beef and pork fat obtained from a butcher. Controls lacking either lipid or culture supernatant were prepared also. The Universals were tightly sealed and incubated horizontally on an orbital shaker (200 rpm for 1 h). The reaction was stopped by adding a solution containing equal quantities of ethanol and acetone (15 ml). The liberated fatty acids were titrated with 0.01 N sodium hydroxide to a pale pink end point using a phelolphthalein indicator.

Protease

Yeasts were inoculated and incubated as previously described for amylase detection (pp64-65) into a modified lab lemco broth containing (g l^{-1}) glucose (BDH, 20) and also a yeast extract (lab m 10g l^{-1}) mycological peptone (lab m 20g l^{-1}) glucose (BDH 20g l^{-1}) broth. Samples were obtained and prepared as previously described.

ASSAY

The ability of yeast culture filtrates to degrade proteins was determined by the method of Cliffe and Law (1982). Samples (1 ml) were pipetted into screw capped Pyrex tubes (12 mm x 7.5 mm) containing 4 ml of a suspension of Hyde powder azure (B grade, Sigma, 15 mg ml^{-1}) in a 0.2 M Tris - HCl buffer (pH 8.3). Positive and negative controls containing trypsin (1 ml,

0.15mg ml^{-1}) and sterile distilled water were prepared also. Incubation was 15 minutes at 30°C on a cyclo-gyrater (40 rpm). The release of the azure dye by proteolytic activity was measured by absorbance (595 nm, Pye Unicam SP6 550 UV/VIS).

Determination of the extent of sulphite binding in pure cultures

Six yeasts isolated from sausage, identified with C. zeylanoides; Cr. albidus var. albidus; D. hansenii; P. membranaefaciens; Rh.rubra; T. candida, were inoculated into Erlenmeyer flasks (250 ml) containing 145 ml of sterile (121°C, 15 min) modified lab lemco broth and incubated for 18 h in a shaking water bath (90 oscillations min^{-1}) at 25°C. The flasks were loosely sealed with cotton wool bungs covered in gauze. Samples (1 ml) of the 18 hour old culture were transferred to 145 ml of sterile medium to which was added 5 ml of a freshly prepared solution of sodium metabisulphite. Controls contained 5 ml of sterile distilled water. During incubation, as noted above, the growth was measured by absorbance (600 nm, Pye Unicam SP8 550 UV/VIS) and related to cell number from a standard curve.

Determination of free and bound sulphite

Samples (1 ml) were withdrawn aseptically immediately after inoculation and at 4 hourly intervals thereafter. The concentration of free and bound sulphite was determined by the method of Banks and Board (1982a, pp 48-50).

Determination of sulphite binding compounds

Acetaldehyde

(1) Steam distillation:

Samples (20 ml) of a stationary phase culture supernatant (4,500 g, 10 min) were steam distilled in a Markham apparatus (Figure 6) into a phosphate buffered (pH 7) sodium metabisulphite (0.5% w/v) ethylene diamine tetra-acetate (EDTA 0.5% w/v) solution. The distillate was acidified by the addition of 25% hydrochloric acid (1 ml) and the excess bisulphite in solution was removed by titration with 0.1 N and 0.01 N iodine to a pale blue end point using a 1% (w/v) starch solution as an indicator. The remaining bisulphite bound to acetaldehyde was liberated by the addition of sodium bicarbonate (5 g) and was titrated with 0.01 N iodine as before. The final titration volume (t) was recorded and related to the concentration of acetaldehyde (c) by the equation :

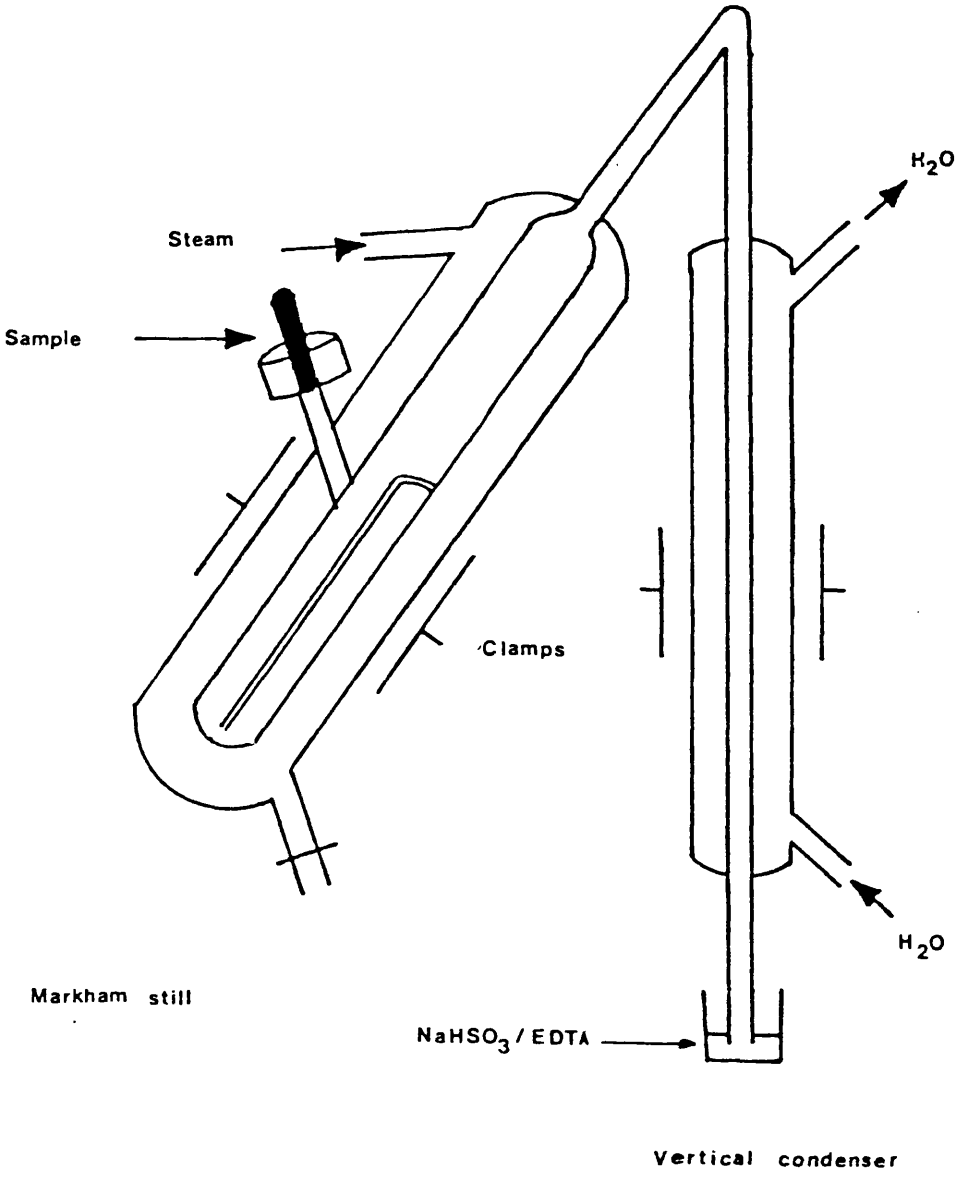
$$c = t \times 11.0$$

Standard solutions of acetaldehyde were measured iodiometrically after steam distillation and direct addition into the sodium bisulphite /EDTA solution, as a reference.

(2) Enzymic

Sample preparation

Figure 6 Diagramatic representation of the Markham distillation apparatus used for acetaldehyde determinations in broth culture.



Samples of stationary phase yeast cultures (1 ml) or sausage (1 g) were first deproteinised in 70% chilled perchloric acid (9 ml) and frozen in liquid nitrogen. The samples were then stored at -20°C until use. The samples were slowly defrosted, shaken and centrifuged (3,000 g 10 min) and the supernatant (6 ml) was added to chilled 0.7 N in potassium phosphate (BDH, 3 ml). The pH of the sample was then adjusted to ca 7.5 with recorded volumes of 2 M chilled sodium hydroxide and centrifuged (3,000 g, 10 min). The supernatant was collected and stored at 4°c in a stoppered Bijoux bottle (5 ml).

ASSAY

The concentration of acetaldehyde in the prepared sample was determined by the enzymic method of Bernt and Bergmeyer (1974a)

PRINCIPLE:

$$CH_3CHO + NADH + H^+ \underset{\text{Dehydrogenase}}{\overset{\text{Alcohol}}{\rightleftharpoons}} C_2H_5OH + NAD^+$$

Acetaldehyde Ethanol

The concentration of acetaldehyde was proportional to the change in extinction at 340 nm arising from NADH oxidation.

PROCEDURE

Samples (2 ml) of deproteinised supernatants were pipetted into micro cuvettes (Sterilin) to which was added a freshly prepared 1.1 mM β reduced nicotinamide adenine dinucleotide (NADH, Sigma) /1% (w/v) sodium bicarbonate solution (0.2 ml) The cuvettes were then sealed with parafilm (Gallenkamp, Dixie, U.S.A.) inverted and the absorbance (E_1) measured with spectrophotometer (Pye Unicam SP6 550 UV - VIS; 340 nm, light path 2 cm; room temperature) against air. A suspension of yeast alcohol dehydrogenase (Sigma, specific activity, 30 mg ml^{-1}) dissolved in 8.2 M ammonium sulphite (0.02ml) was added and the cuvettes were again sealed, inverted and incubated for 15 minutes at 25°C. The final absorbance reading (E_2) was then measured. The concentration of acetaldehyde (c) was then calculated from the equation :

$$c = E_2 - E_1 \times \text{dilution factor} \times 7.86 \ (\mu g \ ml^{-1})$$

Standard acetaldehyde solutions were assayed both directly and also after sample preparation procedures.

<u>α keto glutarate (2 oxo-glutarate)</u>

<u>Sample preparation</u>

This was done as previously described for acetaldehyde.

ASSAY

The concentration of ∝ketoglutarate was determined by the enzyme method of Bernt and Bergmeyer (1974 b).

PRINCIPLE

$$\text{∝ketoglutarate} + \text{NADH} + NH_4^+ + H^+ \underset{\text{dehydrogenase}}{\overset{\text{glutamate}}{\rightleftharpoons}} \text{glutamate} + NAD^+ + H_2O$$

The concentration of ∝keto glutarate was proportional to the change in extinction at 340 nm due to NADH oxidation.

PROCEDURE

The deproteinised sample was pipetted (4.0 ml) into cuvettes (Sterilin) to which a freshly prepared 8.5 mM NADH (Sigma) solution in 1% (w/v) sodium bicarbonate (0.05 ml) was added. The cuvettes were sealed and inverted as before and the absorbance (E_1) was measured (340 nm; light path 2 cm, room temperature; Pye Unicam SP6 - 550 UV/VIS) against air. A suspension of glutamate dehydrogenase (Sigma, 4 mg ml^{-1}) dissolved in 2.0 ml ammonium sulphate solution (0.02 M) was added and the cuvettes were sealed and inverted and the absorbance was measured after 5, 7, 9, 13 and 15 minutes incubation at 25°C. The E_2 value was determined by the extrapolation of these values to the time of glutamate dehydrogenase addition. The small increase in extinction due to the enzyme was determined by adding 0.02 ml of the enzyme

solution at the end of the reaction. The concentration of ∝keto glutarate (c) was given by the equation :-

$$C = E_2 - E \times \text{dilution factor} \times 11.9. \ (\mu g\ ml^{-1})$$

Standard solutions of ∝ketoglutarate were used as a reference as described for acetaldehyde.

Pyruvate

Sample preparation - this was done as described for acetaldehyde.

ASSAY

The concentration of pyruvic acid was determined by using the Boehringer/Mannheim enzymic U/V test combination (cat. No. 124 982)

PRINCIPLE

$$\text{pyruvate} + \text{NADH} + H^+ \overset{\text{LHD*}}{\rightleftharpoons} \text{L-lactate} + NAD^+$$

The concentration of pyruvate is proportional to the change in extinction at 340 nm as a result of NADH oxidation.

* Lactate dehydrogenase

Fermentation in sulphited culture media

The ability of 6 isolates identified with D. hansenii, C. zeylanoides, P. membranaefaciens, T. candida, Rh.rubra and Cr. albidus var. albidus to

ferment glucose in a modified lab lemco broth containing sodium metabisulphite (500 $\mu g\ g^{-1}$ pp 45-46) was investigated as described for the fermentation tests. Control samples lacking sulphite were prepared also.

RESULTS

RESULTS

The microbial association

Analysis of sausages obtained from a factory and retail outlets (18 brands were included in the survey) revealed that in all cases sulphite was present and that the sausage microflora was dominated by Br. thermosphacta, $\geqslant$ lactobacilli and $>$ yeasts (Table 7). Regression analysis (Table 8) showed a high correlation between yeasts, Br. thermosphacta, lactobacilli and total viable counts (as values of >0.348 were found, by using the students "t" test, to be significant, $p =<0.05$, values ca 0.65 were considered, for the purpose of this study, to signify substantial rectilinearity of a relationship). A low correlation existed between these organisms and pseudomonads and enterobacteria. In contrast, analysis of the microbial contaminants in minced beef (180 samples) obtained from butchers and other retail outlets in Bath (Nychas, 1984; Table 9) showed that the correlations between pseudomonad, enterobacteria and the total viable counts were higher than those observed in sausage, but those between yeasts, Br. thermosphacta, lactobacilli and total viable counts were lower. These observations are in accord with those (Dowdell and Board, 1967, 1968, 1971) discussed previously (pp 31-35) and support the view that the British fresh sausage selects a microbial association of yeasts and Gram-positive bacteria, whereas minced meat alone, the principal source of

Table 7 The range of the level of microbial contamination of sausage containing sulphite.

Source of samples	No of samples	TVC	Y	B	L	P	E	Sulphite* ($\mu g\ g^{-1}$) Free	Sulphite* ($\mu g\ g^{-1}$) Bound	pH
		(organisms g^{-1})								
Retail outlets	24 ≠	1.9×10^6	5.0×10^3	1.25×10^5	3.9×10^5	2.5×10^5	1.3×10^5	65-395	105-669	5.2-6.6
		↓	↓	↓	↓	↓	↓			
		6.5×10^{10}	4.7×10^8	2.5×10^{10}	4.0×10^9	1.0×10^7	2.6×10^6			
Factory	7	3.6×10^4	2.0×10^2	1.5×10^4	4.4×10^4	5.2×10^2	4.5×10^3	360-475	80-110	6.2-6.5
		↓	↓	↓	↓	↓	↓			
		4.0×10^7	1.3×10^5	2.0×10^7	3.2×10^{10}	2.5×10^4	4.0×10^4			

* The concentration of free and bound sulphite was determined by the spectrophotometric method of Banks and Board (1982a).

≠ 18 brands were studied

KEY:-

TVC	Total viable count
B	Brochothrix thermosphacta
Y	Yeast
L	Lactobacilli
P	Pseudomonads
E	Enterobacteria

Table 8 Matrix of correlation coefficients (r) between the mean concentrations of microbial contaminants and free and bound sulphite in sausage obtained from retail outlets.*

Yeast	Brochothrix thermosphacta	Lactobacilli	Pseudomonads	Enterobacteria	Free sulphite	Bound sulphite	
0.986	0.810	0.822	0.570	- 0.221	- 0.657	0.413	Total viable count
	0.785	0.729	0.306	- 0.339	0.788	0.691	Yeasts
		0.723	0.553	- 0.131	- 0.326	0.105	Br.thermosphacta
			0.443	- 0.185	0.650	0.354	Lactobacilli
				0.209	- 0.201	0.144	Pseudomonads
					0.112	- 0.198	Enterobacteria
						- 0.598	Free SO_2
						-	Bound SO_2

* number of samples = 31

r values >0.3 significant by student "t" test

r values ca >0.7 considered to indicate greater rectilineality of the regression equation

Table 9 Matrix of correlation coefficients of the mean concentrations of contaminants in minced meat obtained from retail outlets.*+

Yeast	Brochothrix thermosphacta	Lactobacilli	Pseudomonads	Enterobacteria	
0.678	0.748	0.641	0.932	0.652	Total viable count
	0.714	0.430	0.430	0.469	Yeasts
		0.472	0.896	0.611	Brochothrix thermosphacta
			0.501	0.582	Lactobacilli
				0.843	Pseudomonads
				-	Enterobacteria

+ 180 samples studied

* Adapted from the data of Nychas (1984)

r values $>$ 0.1 significant by student "t" test

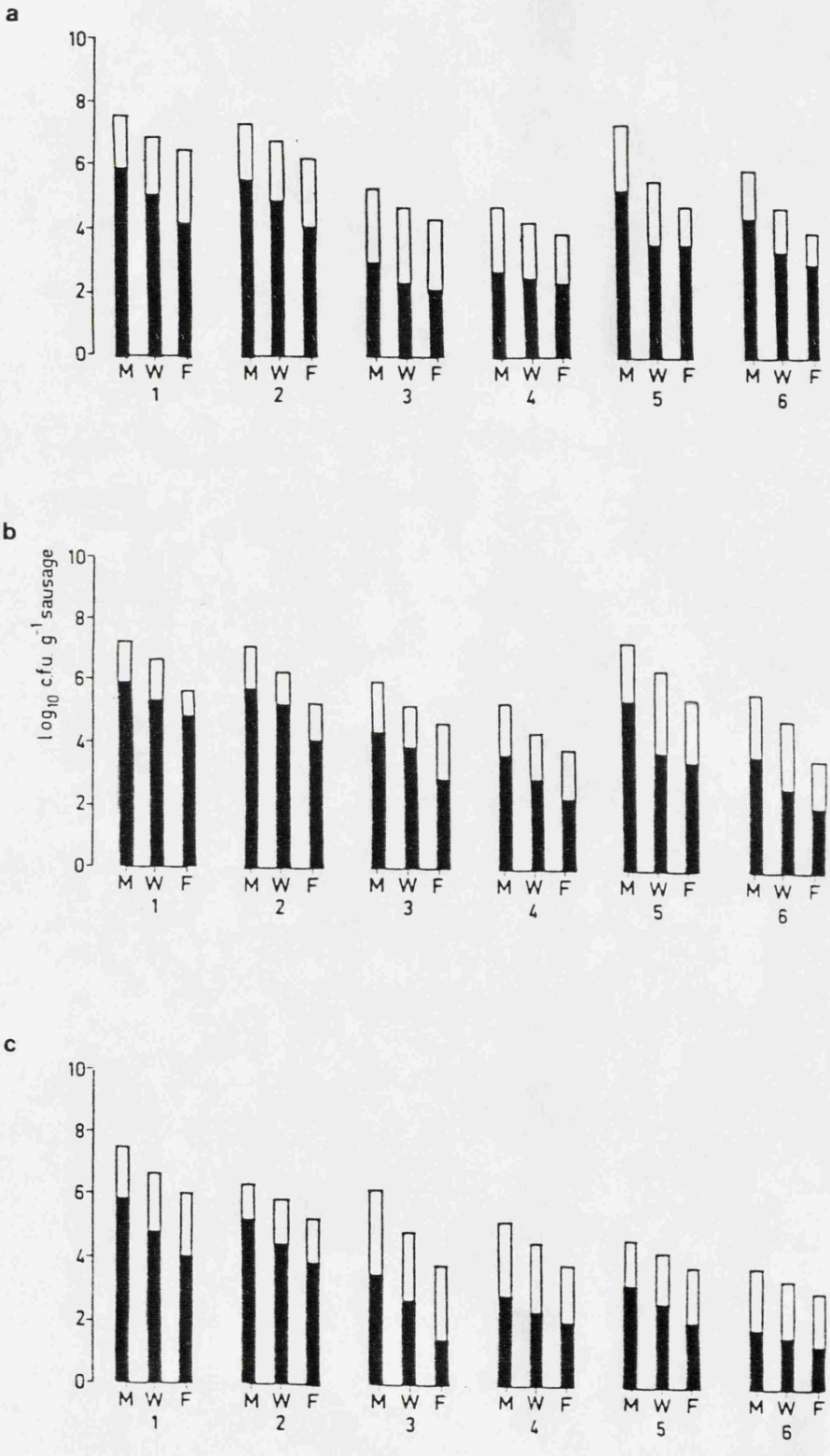
r values $>$ 0.7 considered to indicate greater rectilineality of the regression equation

contaminants of sausage (Ayres, 1960), favours the growth of pseudomonads and enterobacteria.

The concentrations of free and bound sulphite in sausages obtained from retail outlets varied considerably (Table 7). This may have been due in part to different amounts of the preservative in the spice mixtures added to sausages or to different recipes. Storage of the sample before purchase, which has been shown to influence the concentration of sulphite in sausages (Banks and Board, 1982a, pp 9-14), may well have played a part also. In spite of this variation the high correlation that existed between the concentration of bound sulphite and the yeast count ($r = 0.65$) was taken as circumstantial evidence of these organisms' involvement in binding. Table 7 shows that there was a broad range in the numbers of all the microbial contaminants in the British fresh sausage, the range being most extensive in sausages purchased from retail outlets. This was due presumably, at least in part, to the growth of organisms (pp 35-36). The size of the microbial populations recovered immediately following manufacture of pork, pork and beef and pork skinless sausages was influenced by season and also the day of manufacture (Figure 7). Significantly ($p \leq 0.05$) larger populations of yeasts, Br. thermosphacta, lactobacilli, pseudomonads and enterobacteria were recovered from sausages manufactured in Spring (March) than those manufactured in Winter (December). This difference was attributed to the higher

Figure 7 The influence of the day of manufacture on the initial level of microbial contamination in (a) pork; (b) pork and beef and (c) pork skinless sausages.

1	Total viable count	M	Monday
2	Yeast	W	Wednesday
3	*Brochothrix thermosphacta*	F	Friday
4	Lactobacilli	□	Summer
5	Pseudomonads	■	Winter
6	Enterobacteria		


a
b
c
log10 cfu g-1 sausage
0
2
4
6
8
10
M W F
1
2
3
4
5
6

temperature of spring allowing more extensive growth of contaminants on equipment and in ingredients awaiting manufacture. Sausages manufactured on a monday contained significantly ($p =<0.05$) higher microbial populations than those manufactured on Wednesdays and Fridays. Banks (1983) also noted this trend and he attributed it to the use of meat which, through being stored for 48 hours (cf 24 h throughout the rest of the week) over the weekend (ca 5°C), harboured high numbers of micro-organisms. Daily manufacture and routine cleaning of equipment may decrease the level of microbial contamination on working surfaces and be the cause of differences in contamination of sausages produced on Wednesdays (high, $p =<0.05$) and Fridays (low).

Influence of sulphite and temperature on the microbial association of sausage

The fate of sulphite

The concentration of free sulphite diminished rapidly but that of bound sulphite increased when sausages were stored at 4 or 15°C (Figure 8). The concentration of total sulphite diminished only slightly during storage at either temperature. These results, which are in accord with those of Banks and Board (1982) and Banks (1983), support the generally accepted view (pp 9-14) that the reduction in the antimicrobial activity of the preservative during storage is caused by binding rather than catalytic oxidation.

Figure 8 The concentration of free, bound and total sulphite in sausages stored at 4 and 15°C.

○ Free sulphite, 4°C.

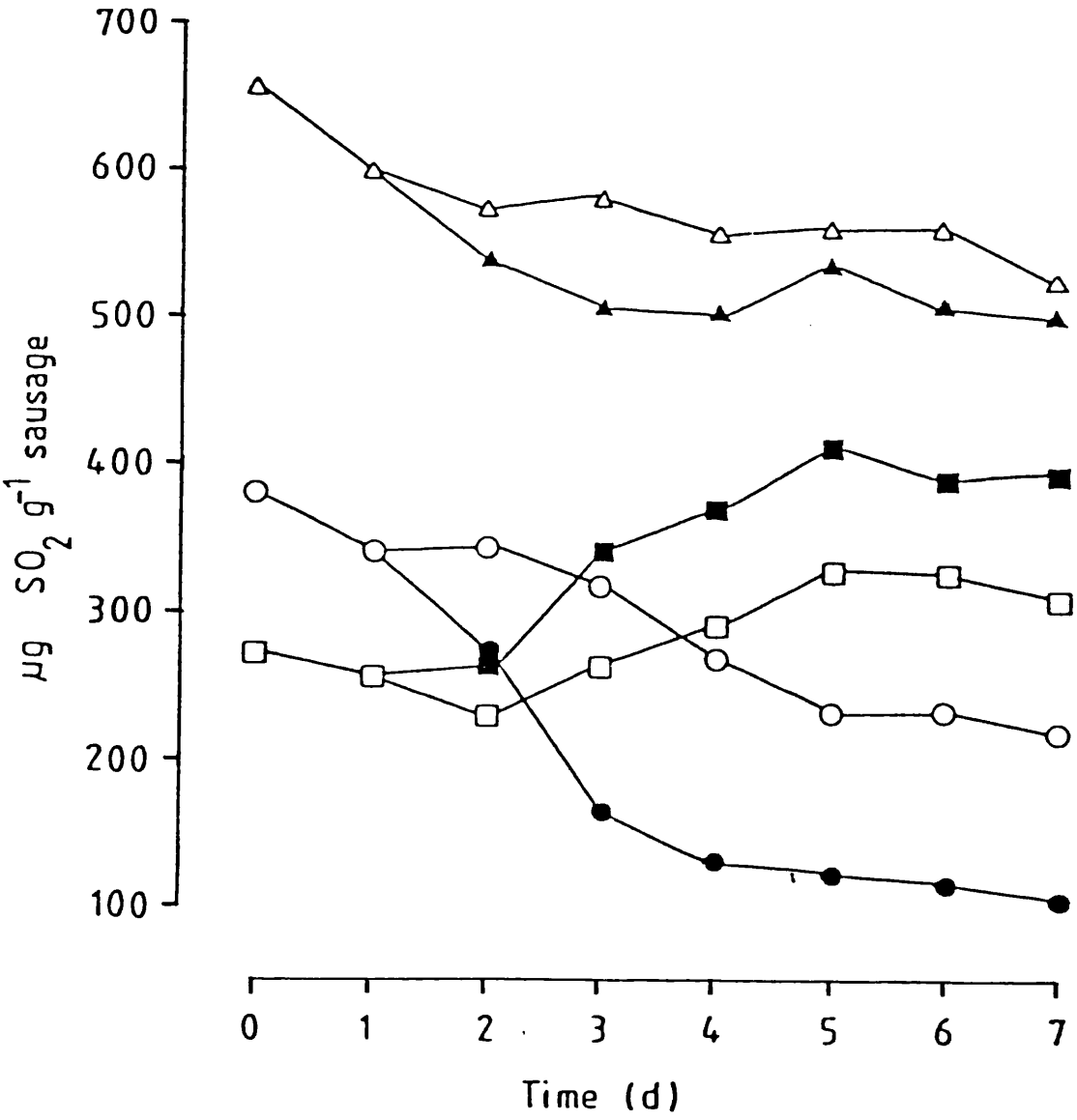
● Free sulphite, 15°C

□ Bound sulphite, 4°C

■ Bound sulphite, 15°C

△ Total sulphite, 4°C

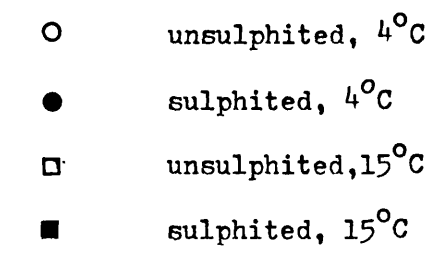
▲ Total sulphite, 15°C



Development of the microbial association

The microbiological examination of sausages stored at 4 and 15°C confirmed the earlier observations of Banks (1983, Table 10), namely that the presence of sulphite reduced significantly (> 0.5 log units; Brown, 1977), the extent and rate of growth (Figure 9a 12a) of those organisms (principally Br. thermosphacta) that grow on PCA (the total viable count). In contrast with other members of the microbial association, the rate and extent of growth of the yeasts (Figure 9b, 12b) appeared to be favoured by sulphite. Indeed, in terms of biomass their populations were such (6.75 and 8.75, $\log_{10}$ c.f.u. g^{-1} at 4 and 15°C respectively) that they could be considered to dominate the microbial association. The temperature of storage appeared to restrict the size of the climax populations but not the growth rate of these organisms. The growth of Br. thermosphacta, initially the numerically dominant organism in all samples (5.0 - 5.5 $\log_{10}$ c.f.u.$g.^{-1}$) appeared to be retarded by sulphite at chill temperatures (Figure 10a 13a). Indeed, larger climax populations were attained in sausages lacking sulphite, especially at 15°C. Similarly the presence of sulphite and/or reduced temperatures of storage retarded the rate and extent of growth of lactobacilli (Figures, 10b, 13b). Sulphite appeared to be most effective against the Gram-negative bacteria, pseudomonads and enterobacteria (Figures 11a, 14a and 11b, 14b respectively); it severely retarded their rates of growth and curtailed the size of their climax populations. These features were pronounced with

Figure 9 The influence of the temperature of storage and sulphite on the growth of (a) general contaminants (Total viable count) and (b) Yeasts in sausage.



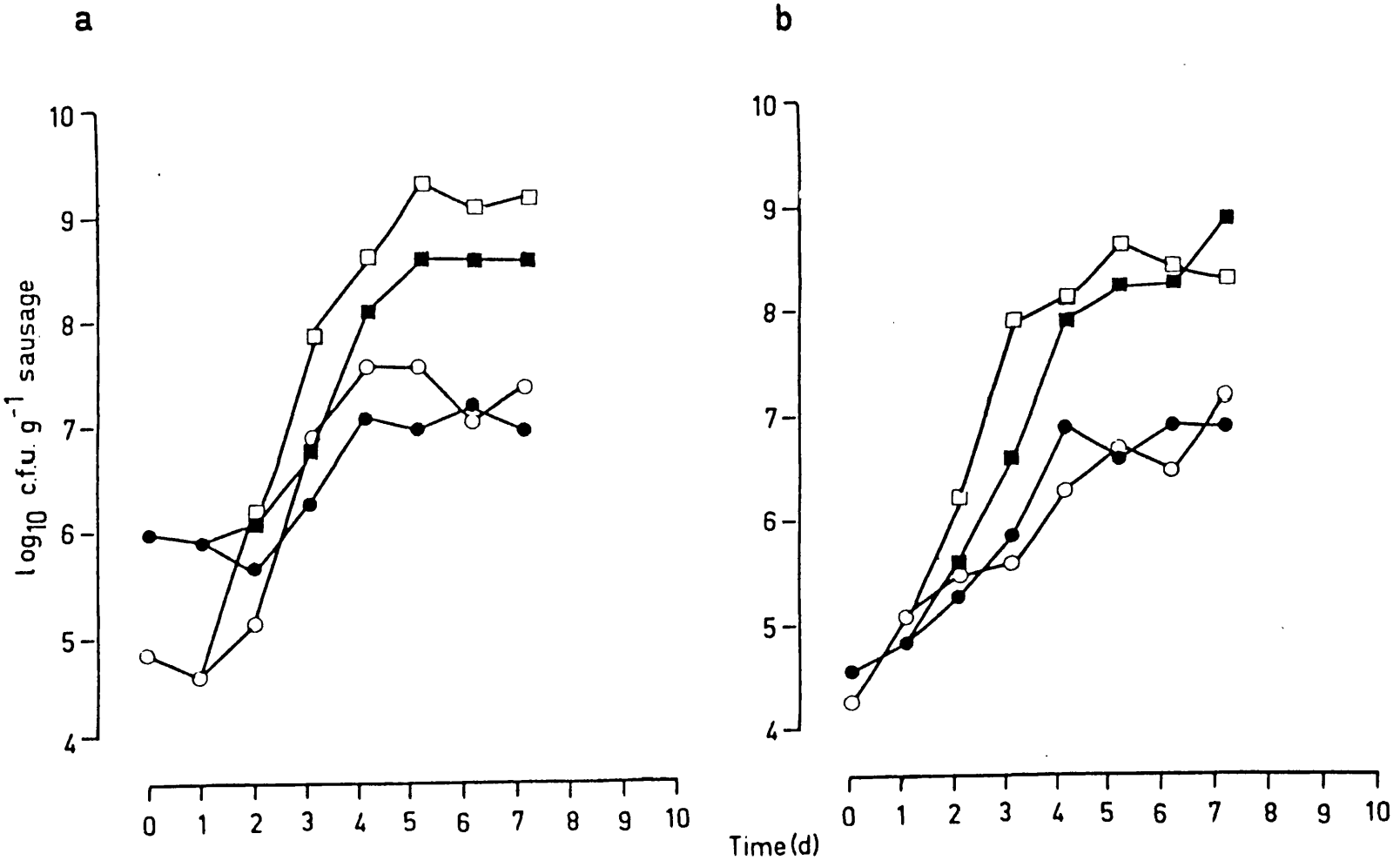

a
b
10
9
8
7
6
5
4
$\log_{10}$ c.f.u. g^{-1} sausage
0 1 2 3 4 5 6 7 8 9 10
Time (d)

Figure 10 The influence of the temperature of storage and sulphite on the growth of (a) Brochothrix thermosphacta and (b) lactobacilli in sausage.

○ unsulphited, 4°C

● sulphited, 4°C

□ unsulphited, 15°C

■ sulphited, 15°C

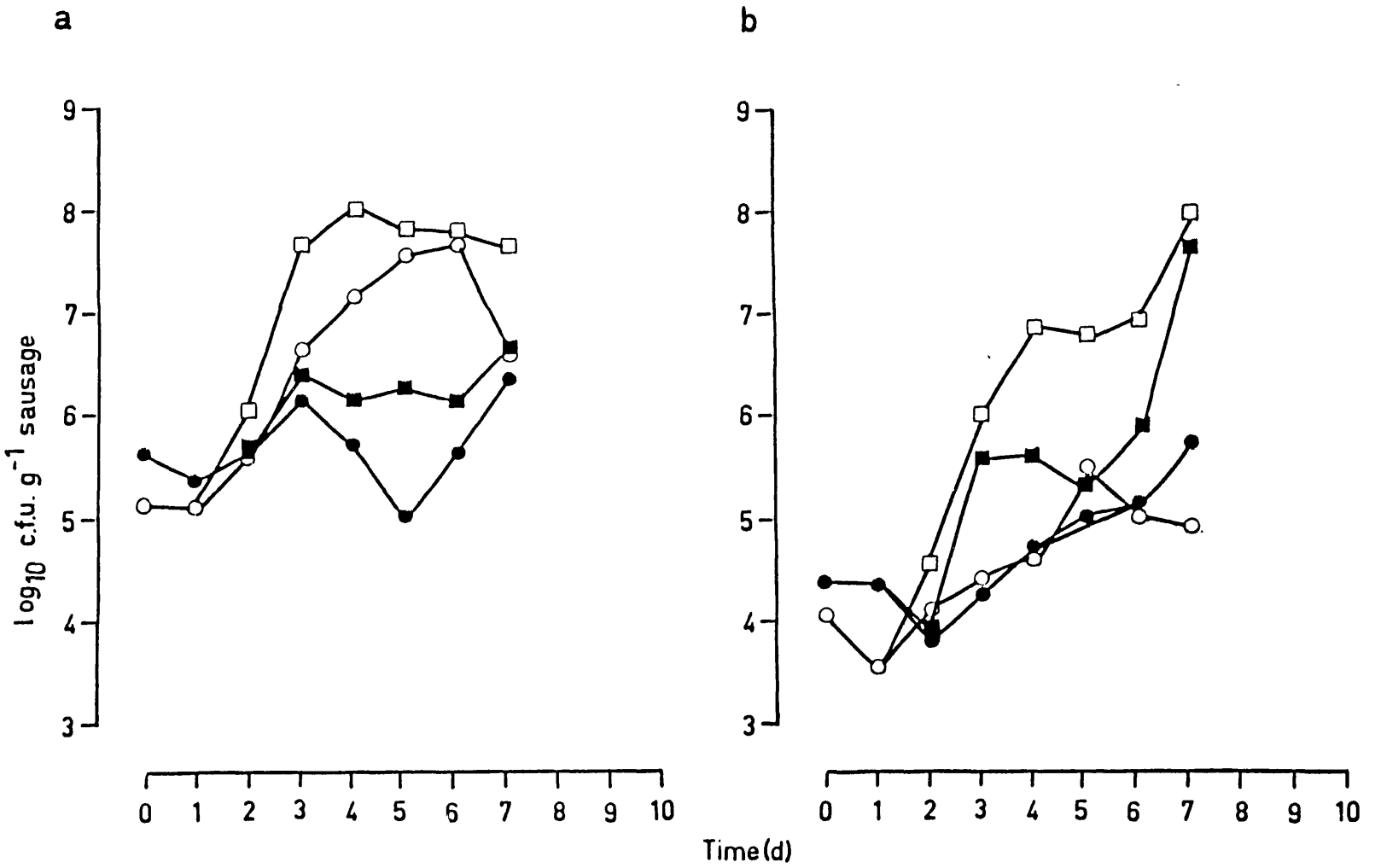
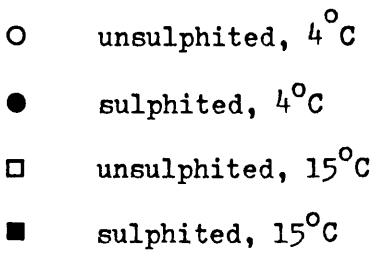

a
b
9
8
7
6
5
4
3
log10 c.f.u. g-1 sausage
0 1 2 3 4 5 6 7 8 9 10
Time (d)

Figure 11 The influence of the temperature of storage and sulphite on the growth of (a) Pseudomonads and (b) Enterobacteria in sausage.



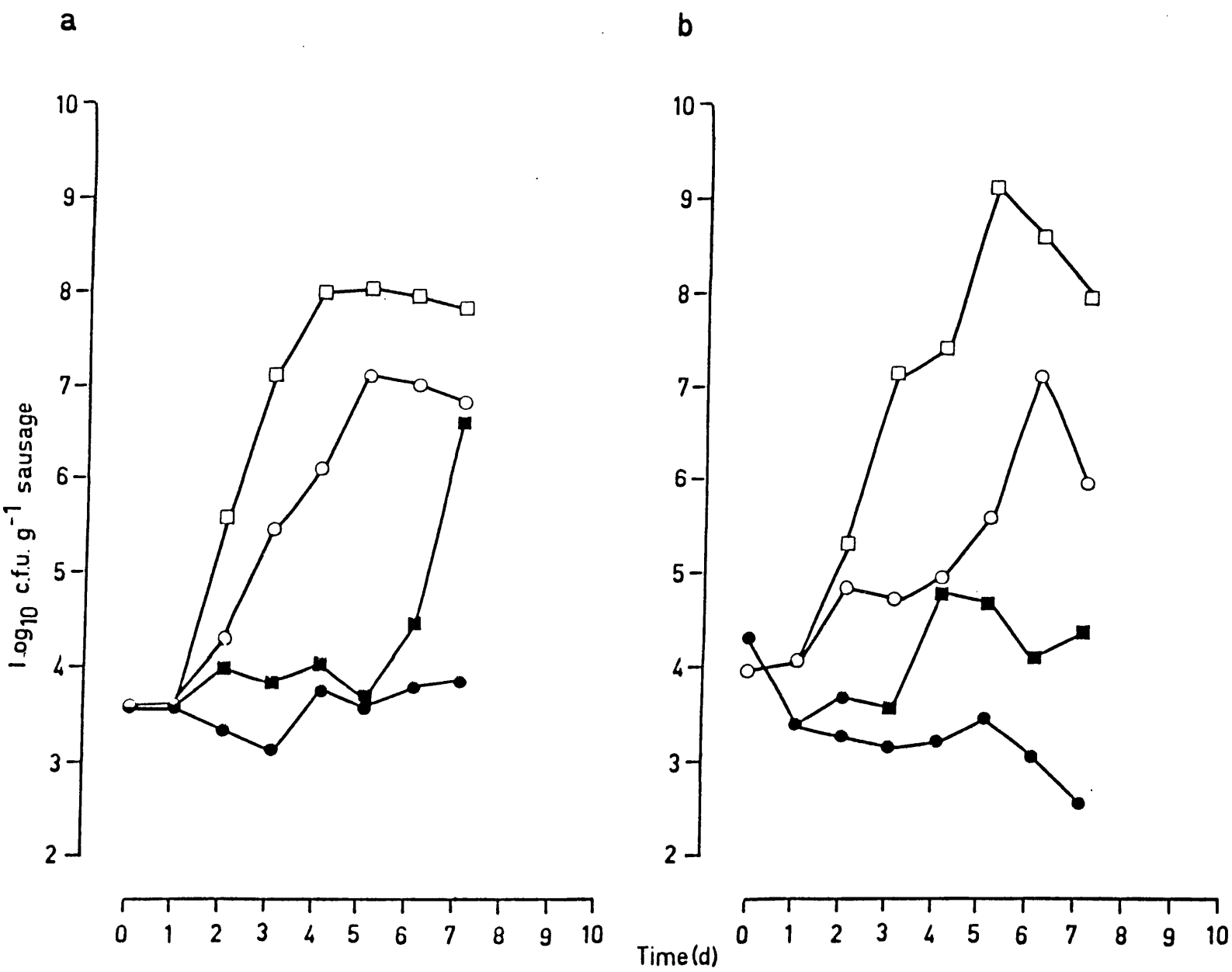
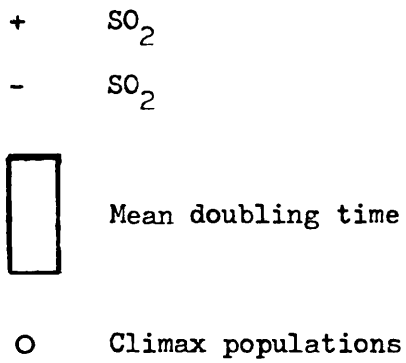
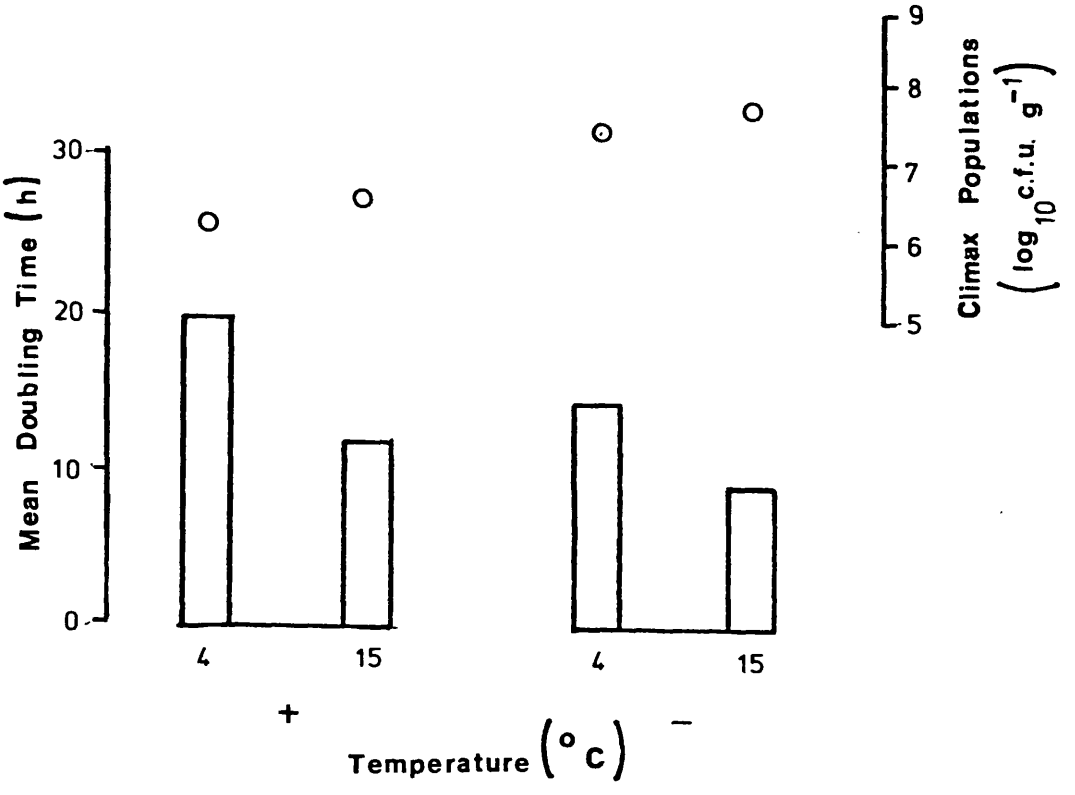

a
b
10
9
8
7
6
5
4
3
2
log10 c.f.u. g-1 sausage
0 1 2 3 4 5 6 7 8 9 10
Time (d)

Figure 12 Influence of the temperature of storage and sulphite on the rate (mean doubling time) and extent (climax populations) of (a) general contaminants (Total viable count)and (b) Yeasts in sausage.



a


Mean Doubling Time (h)
30
20
10
0
4
15
4
15
+
Temperature (°C)
−
Climax Populations (log10 c.f.u. g-1)
9
8
7
6
5

b

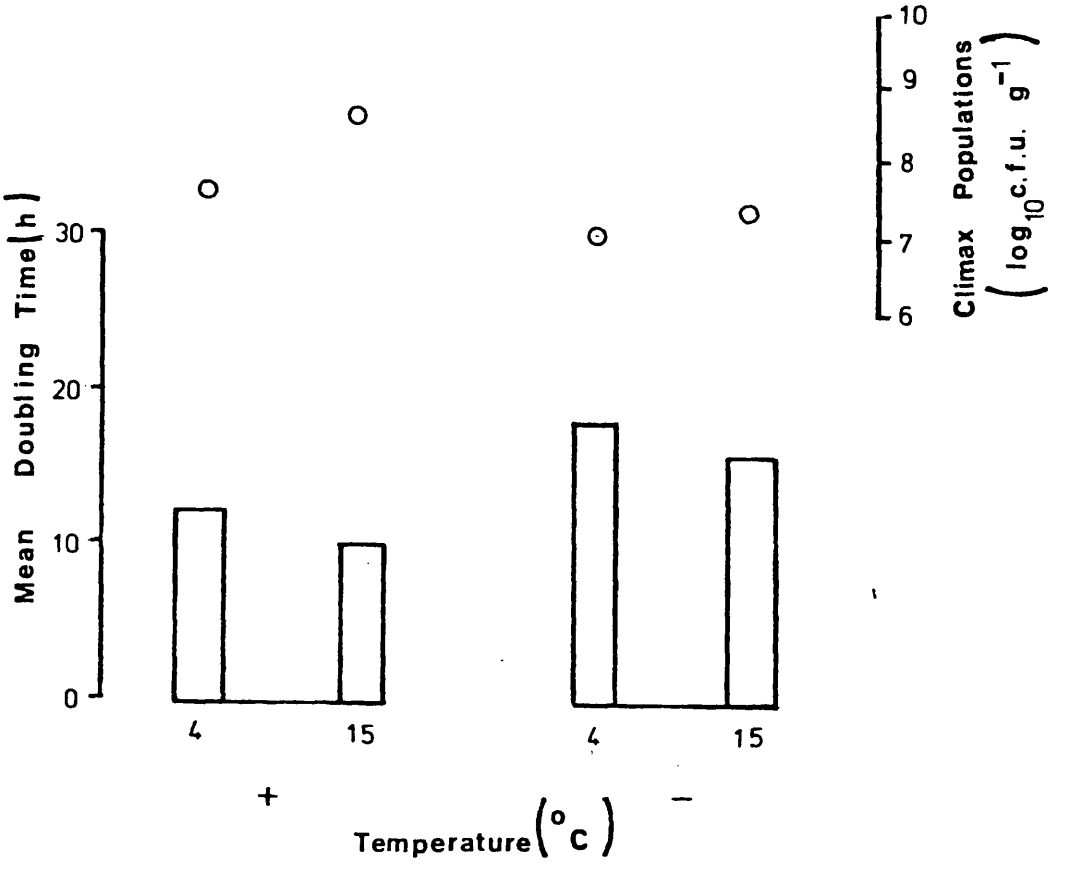
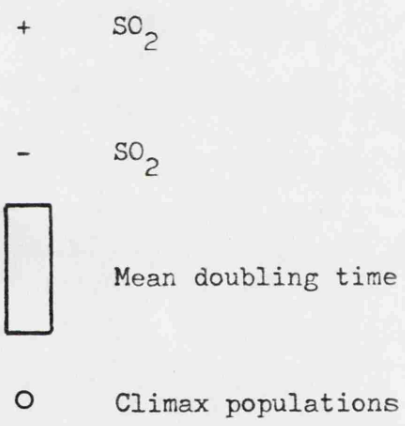
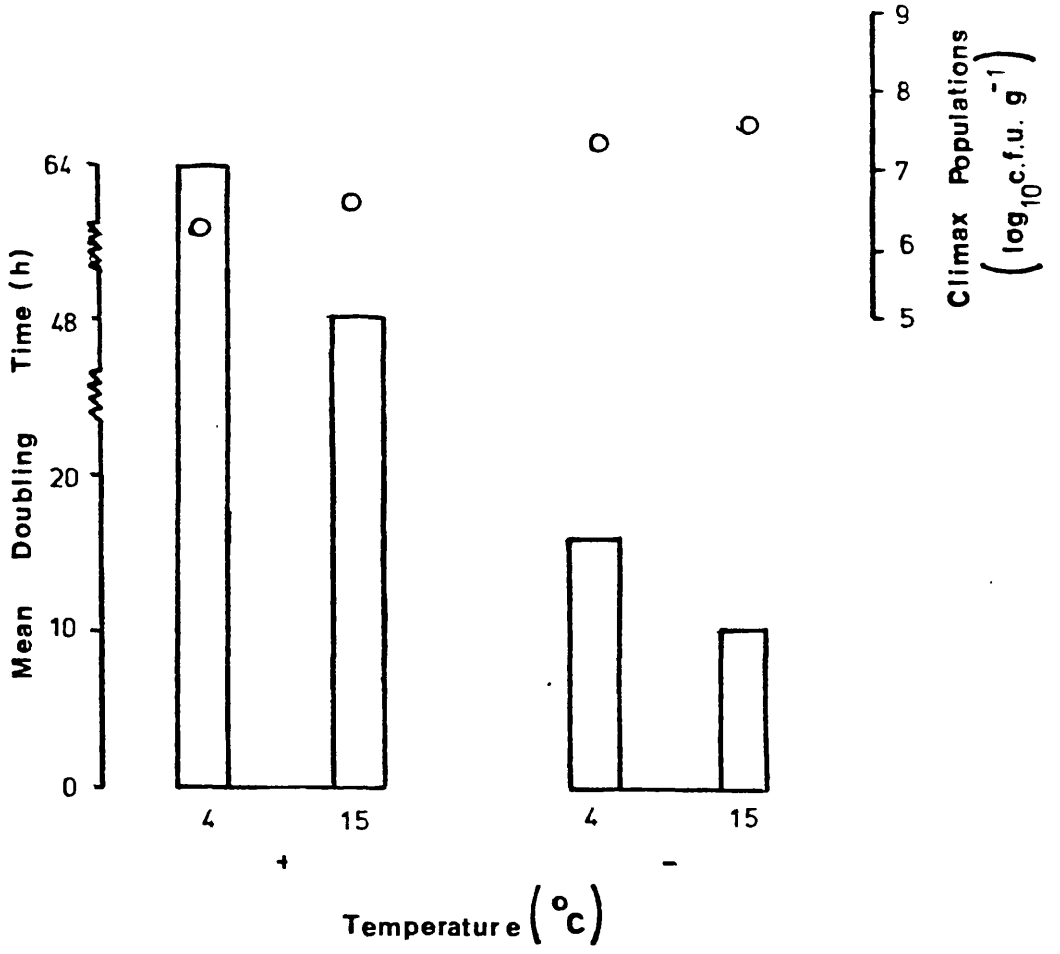

Mean Doubling Time (h)
30
20
10
0
4
15
4
15
+
Temperature (°C)
−
Climax Populations (log10 c.f.u. g-1)
10
9
8
7
6

Figure 13 The influence of the temperature of storage and sulphite on the rate (mean doubling time) and the extent (climax populations) of growth of (a) Brochothrix thermosphacta and (b) Lactobacilli.



a


Mean Doubling Time (h)
64
48
20
10
0
4
15
+
4
15
-
Temperature (°C)
Climax Populations
(log10 c.f.u. g-1)
9
8
7
6
5

b

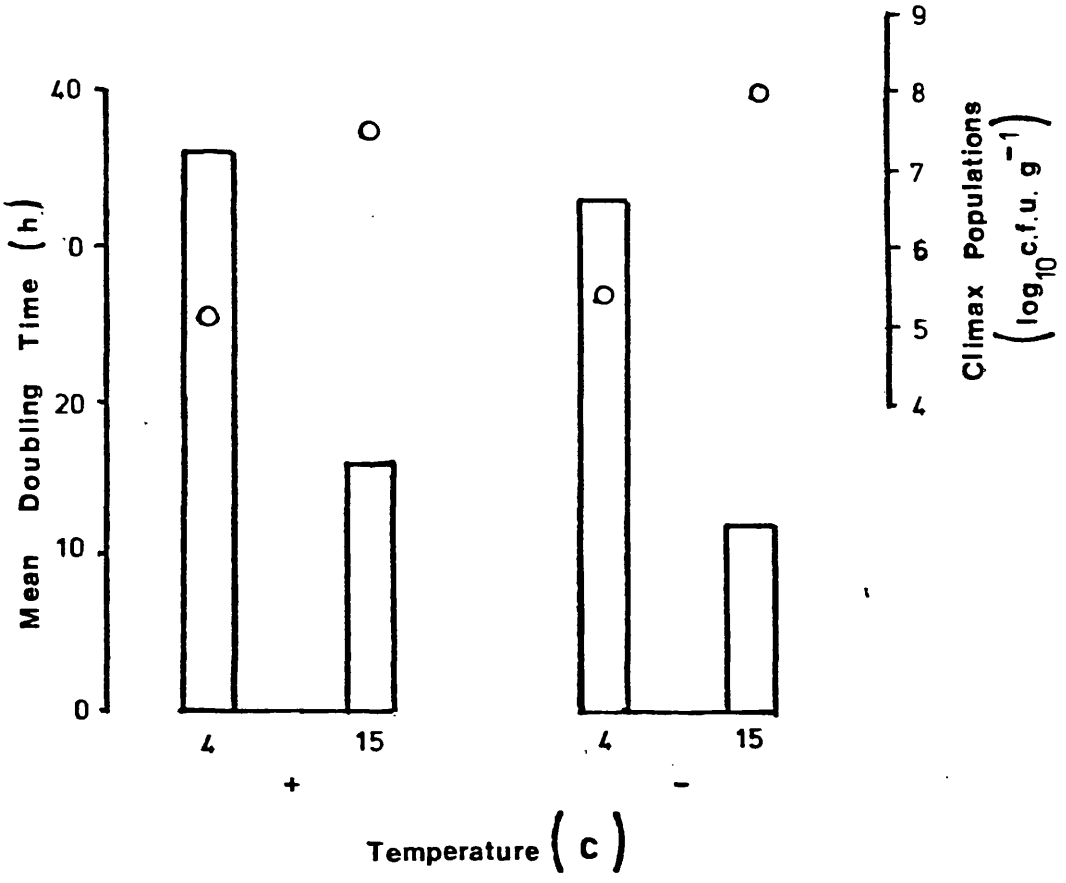
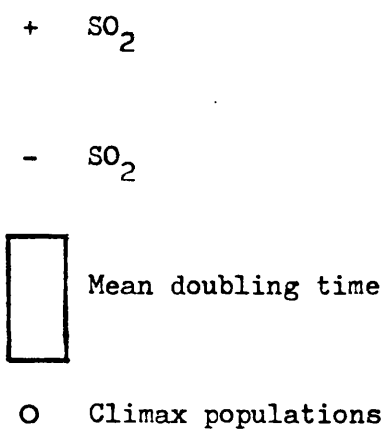
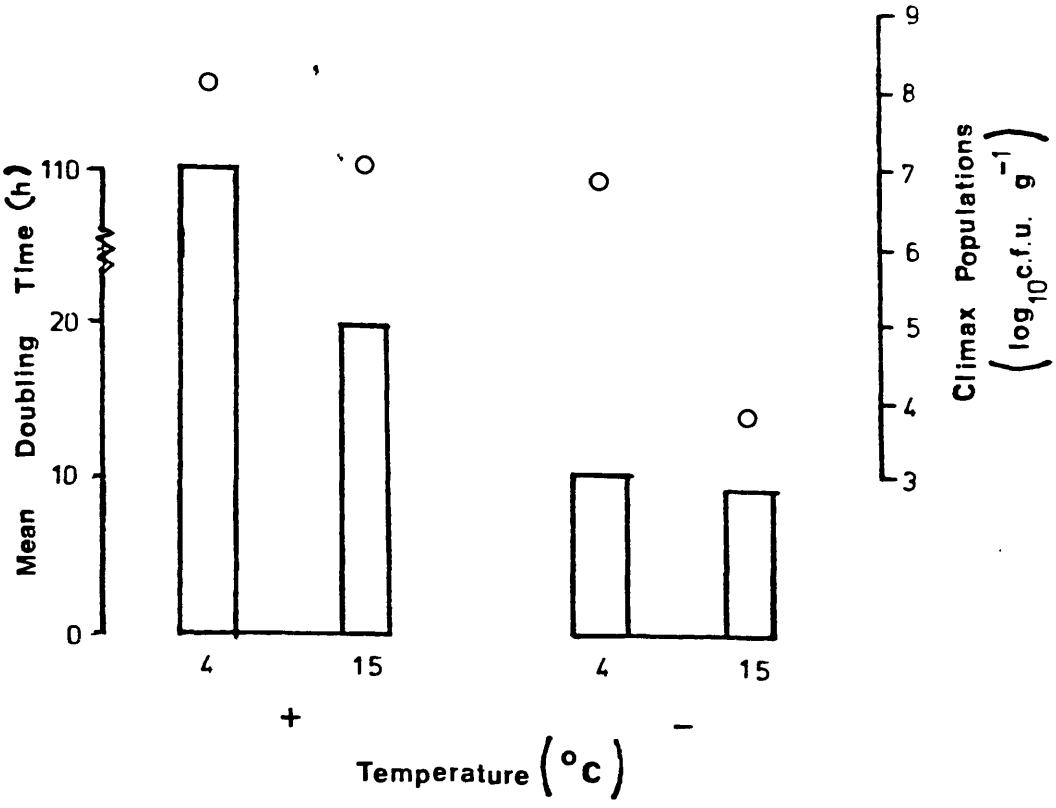

Mean Doubling Time (h)
40
0
20
10
0
4
15
+
4
15
-
Temperature (C)
Climax Populations
(log10 c.f.u. g-1)
9
8
7
6
5
4

Figure 14 Influence of the temperature of storage and sulphite on the rate (mean doubling time) and extent (climax populations) of growth of (a) Pseudomonads and (b) Enterobacteria.



a


Mean Doubling Time (h)
110
20
10
0
4
15
4
15
+
−
Temperature (°C)
9
8
7
6
5
4
3
Climax Populations (log10 c.f.u. g-1)

b

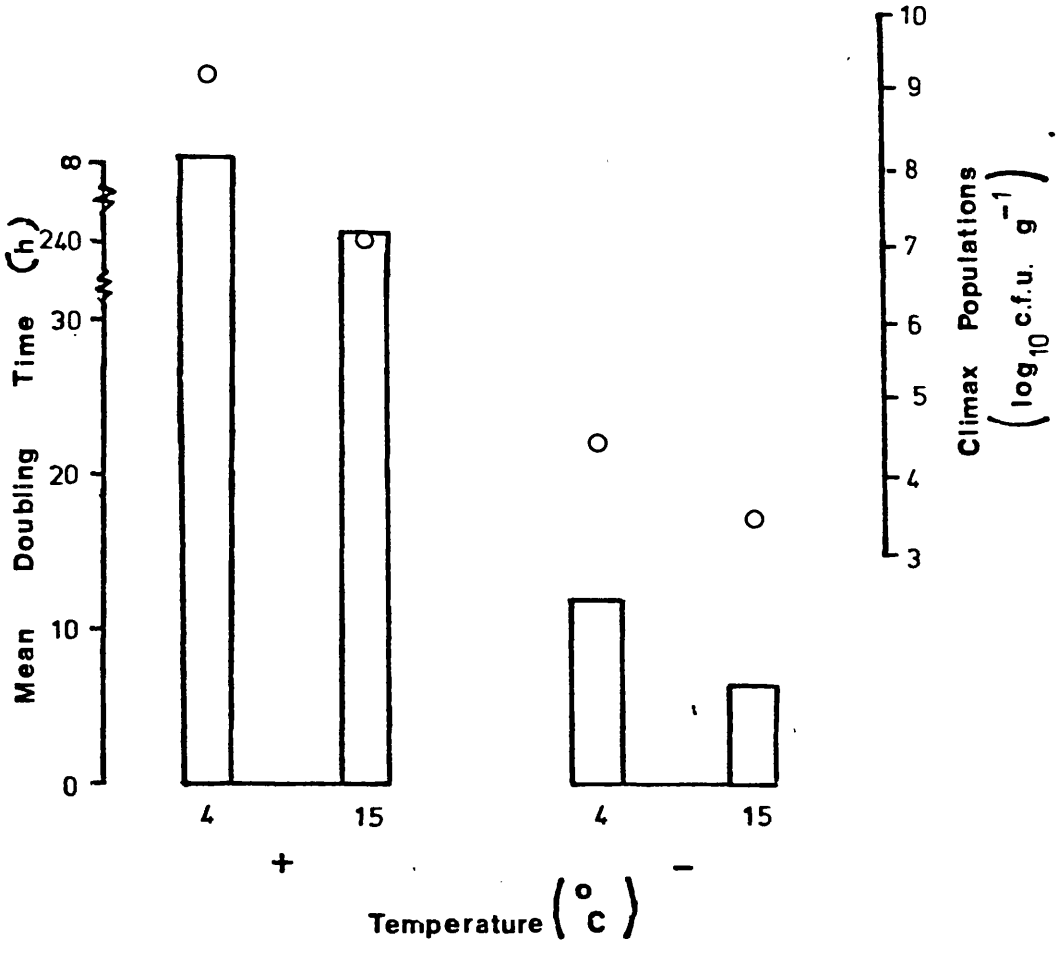

Mean Doubling Time (h)
240
30
20
10
0
4
15
4
15
+
−
Temperature (°C)
10
9
8
7
6
5
4
3
Climax Populations (log10 c.f.u. g-1)

Table 10 Influence of temperature and sulphite concentration on the growth of contaminating micro-organisms in sausages dominated by Brochothrix thermosphacta [1] or yeasts [2*]

Organism	Initial Concentration	Climax Populations (log_{10} c.f.u.g.$^{-1}$)					Mean doubling times (h)				
		4≠		10	15		4		10	15	
		1	2	1	1	2	1	2	1	1	2
Total viable organisms	+ ng	8.5	ng	9.5	9.0	ng	18.0	ng	8.0	9.0	ng
	- ng	9.0	ng	10.0	10.7	ng	15.0	ng	7.0	6.0	ng
Yeast	+ 3.2 - 4.9	6.1	7.4	7.0	7.5	8.4	17.0	18.0	13.0	10.0	17.0
	- 2.5 - 4.7	5.5	7.0	7.2	7.8	9.0	20.5	13.0	13.5	8.0	12.0
Brochothrix thermosphacta	+ 4.3 - 7.9	8.0	6.4	8.5	8.0	6.5	23.0	63.0	19.0	11.5	46.0
	- 4.7 - 8.2	9.0	7.5	9.2	8.5	7.6	11.5	17.0	9.0	8.5	15.0
Lactobacilli	+ 2.2 - 5.3	2.5	5.0	3.0	8.0	7.5	25.0	31.0	72.0	11.0	17.0
	- 2.3 - 6.4	5.0	5.5	7.0	8.5	8.5	19.0	26.0	10.0	7.0	13.0
Pseudomonads	+ 2 - 6.13	4.5	4.0	6.5	5.0	6.0	360.0	101.0	15.8	22.0	20.0
	- 2 - 5.89	6.3	7.0	6.3	6.75	8.0	10.0	11.0	9.0	8.5	9.0
Enterobacteria	+ 2.4 x 4.4	3.3	4.0	3.7	5.2	4.5	43.0	∞	24.0	21.0	150.0
	- 3.5 - 4.9	6.2	6.5	9.8	9.2	9.0	8.0	14.0	5.0	4.5	9.0

* Adapted from the data of Banks (1983)

≠ Storage temperature (°C)

\+ Sulphited sausages

\- Unsulphited sausages

storage at 4°C. As noted by Banks (1983) the populations of the pseudomonads increased rapidly on the 6th and following days of storage at 15°C at which time the concentration of free sulphite had fallen below 120 μg g^{-1} sausage (Figure 8). Enterobacteria are considered to be the most sulphite-sensitive contaminants of sausages. In this study they remained quiescent in sulphited sausages at 4°C. In the absence of sulphite the rate of growth of the pseudomonads and, to a greater extent, the enterobacteria and the climax populations which both attained were influenced by the temperature of storage, the smallest populations being formed at 4°C. The findings outlined above are in accord with those of Banks (1983).

Influence of sulphite and temperature on pH

The pH (initial value, ca 6.2 Figure 15) of sulphited sausage did not change appreciably during storage but there was an acid drift of about 1 pH unit in unsulphited ones stored at 15°C. These findings are in agreement with those of Banks (1983).

In general, my observations indicate that the microbiology of the sausages sampled in this survey can be considered to be "typical" of those studied by other workers (pp 31-35).

Identification of yeasts

This phase of the study was undertaken with the objective of

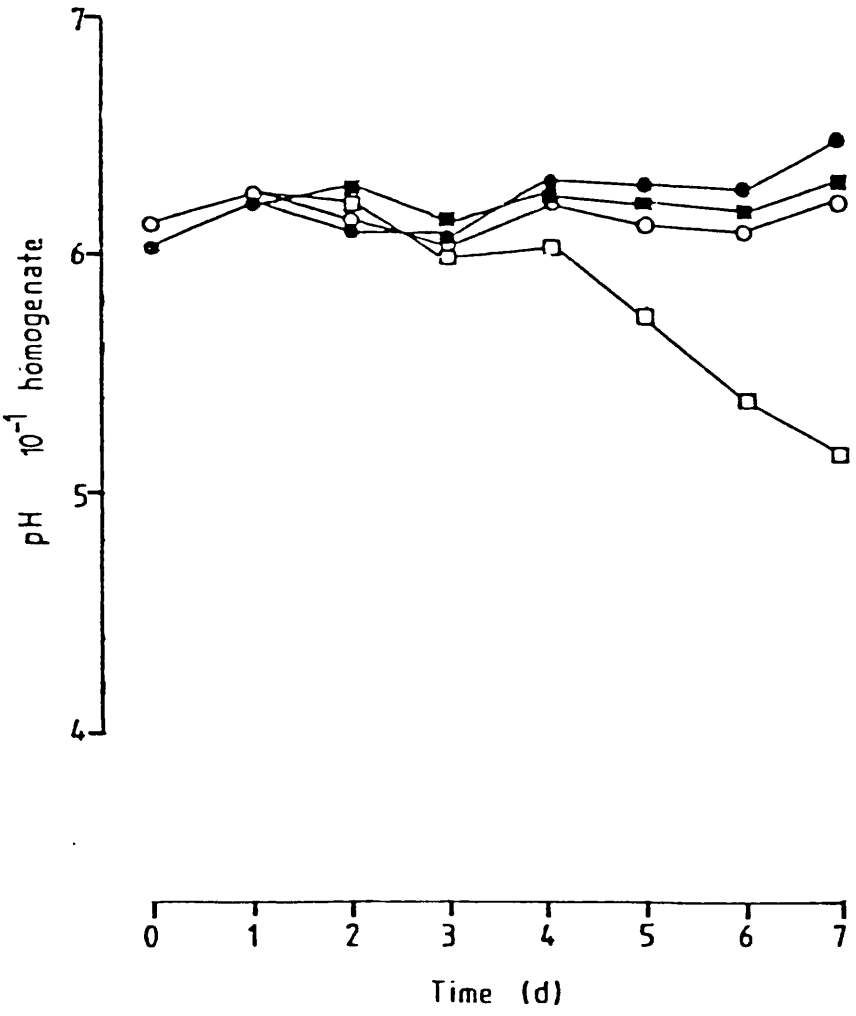
Figure 15 The pH of sulphited and unsulphited sausage homogenates stored at 4 and 15°C.

○ unsulphited, 4°C

● sulphited, 4°C

□ unsulphited, 15°C

■ sulphited, 15°C



identifying the principal yeast contaminants of sausages. A total of 195 yeast isolates from 37 sulphited and 40 isolates from 7 unsulphited samples of pork sausage were studied. As minced beef from butchers shops and retail outlets in Bath contained appreciable numbers (6.4×10^3 - $5.0 \times 10^7 g^{-1}$) of yeasts (Nychas, 1984), 95 isolates from this source were studied also with the objective of establishing whether or not the constant use of sulphite in a sausage factory elected a particular yeast flora. A further 54 yeast isolates from skinless sausages sampled by Fielder (1983) were studied with the objective of establishing whether or not the heat treatment during processing (pp 5, 37-40) had an elective action on the yeast flora.

(1) Taxonomy

A total of 384 yeasts randomly selected in the course of the studies discussed on (pp 76-92) were characterised morphologically (Table 11) and biochemically (Table 12) and identified (Table 13) with the definitions of (Lodder, 1970). A list of these species and their synonyms (Barnett et al., 1983) are given in the Appendix. Sixty six isolates were fully characterised on two occasions, as is recommended by Sneath and Johnson (1972), to determine the extent, if any, of within laboratory variations in the tests. A percentage difference in the two sets of results of 1.25 was observed, which is well below the 5%

Table 11 Morphological and microscopic characteristics of the yeast species recovered from sausage, ingredients and minced beef.

Species	Morphology ≠	Microscopy ø	
		size (µm)	cell characteristics
Bullera alba	Cr.Sm.Sh	(3.5 - 4.5) x (4.0 - 5.0)	O.Bl (singular)
tsugae	G.Sm.Sh.M*	(2.3 - 4.5) x (3.0 - 6.5)	O (singular)
Candida albicans	Cr.Sm.Sh. MP*	(3.5 - 6) x (6.7 - 9.5)	O (chains), E, PM, Bl.
ciferrii	Cr.Wr.H.MP.	(3.0 - 4.5) x (4 - 10)	O (chains), TM. Bl.
curvata	B.M.Sm./Y.M.D.	(2.0 - 3.1) x (6 - 9.6)	O. Cy. PM.
foliarum	Cr. Sm. Sh.	(1.5 - 4) x (4 - 12)	Cy.E. PM (chains of cells)
humicola	Y.Sm.M/Wr.D	(3.5 - 5) x (8 - 30)	O. Ap. Cy. PM. TM. Bl.
ingens	Cr.D.Wr.	(4 - 8) x (8 - 16)	O. Cy. PM. (chains)
lipolytica var. deformans	Cr.Wr.Sh.	(3 - 5) x (5 - 17)	O. E. PM. TM. B. (terminal)
lipolytica var. lipolytica	Cr.Wr.Sh.	(3 - 5) x (5 - 17)	O. E. PM. TM. B. (terminal)
mesenterica	Cr. D.H.* MP.	(2 - 4.5) x (6 - 12)	O.E. PM.
ravautii	Cr.Sm.Wr.* MP.	(2.5 - 4) x (6 - 9)	O. Cy. PM.Bl.*

continued

Table 11 continued

Species	Morphology ≠	Microscopy ø size (µm)	cell characteristics
Candida			
rugosa	Cr/B. Wr. D	(2.5 - 4) x (7 - 15)	O. E. Cy.*
sake	Cr. Sm. D.	(2.5 - 5) x (4 - 7)	O. g. PM. Bl
silvae	Cr.SM.Sh. D*	(1.5 - 4) x (2 - 6)	O. g. PM. Bl. (clusters)
valida	Cr./G.D. Wr*	(2 - 4) x (4 - 10)	O. Cy. PM (branched)
vini	Cr.D.Sm.	(2 - 5) x (3 - 9)	O. Cy. PM (branched)
zeylanoides	Cr.D.Sm./Wr.MP	(2 - 4) x (4 - 9)	O. PM. Bl (bunches)
Cryptococcus albidus var. aerius	Cr./B.M.	(3 - 7) x (4 - 9)	O. g. C.
albidus var. albidus	Cr.Sm.Sh.	(3 - 6) x (4 - 9)	O. g. C.
albidus var. diffluens	Cr./B.M.	(3 - 6) x (4 - 9)	O. g. C.
dimennae	B./P. M.	(2.8 - 6.8) x (3.5 - 2.8)	O C*
gastricus	B.M.	(3.0 - 7.0) x (5.5 - 9.5)	g.
hungaricus	R.M.	(4.5 - 8) x (5 - 8.7)	O. g.
laurentii var. flavescens	Cr. M.	(2 - 5) x (3 - 7)	O. E.

continued

Table 11 continued

Species	Morphology ≠	Microscopy ø size (µm)	Microscopy ø cell characteristics
Cryptococcus			
laurentii var. laurentii	P./B. M.	(2 - 5) x (3 - 7)	O.E.
laurentii var. magnus	P./B. M.	(2 - 5) x (3 - 7)	O. E.
macerans	R.Sm.D.	(3 - 4.5) x (5 - 11)	O.E.
skinneri	B./Cr.M.	(2.6 x 5.2) x (4.5 - 7.2)	O. g.
uniguttulatus	Cr.Sm.Sh.	(3.0 - 5.2) x (3.5 - 7)	O. g.
Debaryomyces hansenii	W.Sm.D.	(2 - 7) x (2 - 7)	C. A.
marama	Cr. D.	(2 - 4) x (3.5 - 9)	O. A.
Hansenula polymorpha	Cr.Sm.Sh.	(1.4 - 3.5) x (2.1 - 8.6)	Cy. A.
Leucosporidium capsuligenum	Cr.Sm.D. MP*	(2.5 - 10.1) x (4.0 x 16.8)	O. g. Pm. (branched)
scottii	Cr.M.MP.	(1.3 - 6.7) x (4.0 - 16.1)	O. E. PM. B.
Pichia etchellsii	Cr.Sm.Sh.	(2.5 - 5.8) x (4 - 11)	O. A. PM.
media	Cr.Sm.Sh.	(2 - 3.1) x (3 - 5.5)	Ov. (chains) A.
membranaefaciens	Cr./B.Sm.D.Wr.*	(2 -4.5) x (5 - 2.0)	O. Cy. PM. (Treelike) A.

continued

Table 11 continued

Species	Morphology ≠	Microscopy ø size (µm)	Microscopy ø cell characteristics
Pichia			
vini var. melibiosi	Cr.Sm.D.Wr.	(1.5 - 4.5) x (14 - 18)	O. Cy. P.M. (branched) A
vini var. vini	Cr.Sm.D.Wr.	(1.5 4.5) x (14 - 18)	O. Cy. P.M. (branched) A
Rhodotorula glutinis	R.M.	(2.3 - 5) x (5 - 16)	O. g.
graminis	R.M.	(2.5 - 4) x (4 - 10)	O. g.
marina	P.Sm.Sh.	(2.5 - 5) x (3 - 12)	g. E.
minuta var. minuta	P. Sm.Sh.	(2.3 - 4.5) x (7 - 11)	O. g.
pallida	P.Sm.Sh. Wr*	(3.0 - 5.5) x (40 - 70)	O. g.
rubra	P./R.M.	(2.5 - 6) x (4.6 - 11)	O. E.
Torulopsis candida	Cr.Sm.D. Wr*	(2 - 7) x (4 - 85)	C. O.
domercqii	Cr.Sm.Sh.	(1.5 - 3) x (1.5 - 2.3)	O. g.
inconspicua	Cr.Sm.D.	(3 - 55) x (5.5 x 7.5	O.
norvegica	Cr.Sm.Sh.	(1.5 - 3) x (2.5 - 5)	O.

continued

Table 11 continued

Species	Morphology ≠	Microscopy ø	
		size (µm)	cell characteristics
Torulopsis vanderwaltii	Cr.Sm.Sh.	(2 - 3.1) x (5 - 6)	Ou. Cy.
Torulopsis versatilis	Cr.Sm.Sh.	(2.5 - 3.5) x (3 - 4.5)	O. g.
Trichosporon cutaneum	Cr.Wr.MP. (extensive)	(3.5 - 7) x (3.5 - 14)	O.E. PM. TM. Ar.

KEY :-

* Rarely expressed attributes

Morphology

B	Beige	MP	Mycelial perimeter
Cr	Cream	P	Pink
D	Dull	R	Red
G	Grey	Sh	Shiny
H	Hairy	Sm	Smooth
		W	White
M	Mucoid	Wr	Wrinkled

Microscopy

Ap	Apiculate	Cy	Cylindrical
Ar	Arthrospores	E	Elongated
A	Ascospores	g	Gl obose
C	Circular	O	Ovoid
Cl	Chlamydospores	PM	Pseudomycelium
Bl	Blastospores	TM	True Mycelium

≠ Streak cultures on malt extract agar (incubation 25°C for 3 weeks) were examined.

ø Microscopic examination of cell suspensions from malt extract cultures and streak cultures of isolates on corn meal agar (incubation 3 days at 25°C and 4 days at 25°C respectively).

Table 12 Biochemical activities of the yeast species isolated from sausage, ingredients, minced beef and a local factory.

Species	No.	Isolates	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	41	41	42
			x			x								x		x							x		x									x									
Bullera alba	1*		0	0	1	1	0	1	1	0	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	0	1	1	1	0	1	0	1	0
teugee	2		0	0	0	0	2	2	2	2	0	0	2	2	2	0	2	0	0	0	2	2	0	2	1	0	2	0	0	0	0	0	2	0	2	0	2	2	2	0	0	2	2	0
Candida albicans	2		2	0	0	0	0	0	1	2	0	2	2	0	2	0	2	0	0	0	0	2	2	2	0	0	1	0	0	0	1	0	0	2	2	2	2	2	2	0	2	0	0	2
ciferrii	8	8	0	4	16	16	1	16	16	16	16	16	16	13	16	1	16	16	0	16	0	16	6	16	6	16	0	0	16	15	16	16	16	7	16	14	16	16	16	0	16	0	14	16
curvata	4	18	0	0	0	7	4	22	9	22	22	22	12	0	22	0	22	22	0	22	22	22	4	20	4	0	16	21	22	18	20	22	22	4	18	10	20	22	22	0	22	0	21	19
foliarus	1		0	0	0	0	0	1	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	1
humicola	11	7	0	2	18	11	0	18	18	18	18	18	15	1	18	1	18	18	0	18	18	18	8	18	11	18	14	18	4	18	17	18	18	11	15	14	13	17	18	2	18	0	14	15
ingens	2		0	0	0	0	0	0	0	2	0	2	0	0	2	0	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	0	0	0	0	0	0	1
lipolytica var deformans	7		0	3	0	0	3	7	7	7	7	0	0	2	7	0	7	0	0	7	0	0	7	7	7	0	0	0	0	0	0	4	7	5	6	0	7	0	0	0	0	0	7	0
lipolytica var lipolytica	8	9	0	4	0	0	3	0	17	17	17	0	0	0	17	0	17	0	0	17	0	0	5	17	5	0	0	0	0	0	0	0	0	5	15	0	17	0	0	0	0	0	15	11
mesenterica	21		0	2	0	0	9	20	21	21	21	0	21	6	21	2	21	0	0	21	0	21	17	21	18	0	5	21	0	0	15	10	20	20	21	0	21	21	21	0	0	0	15	0
reversii	8	1	0	1	0	0	0	0	9	9	0	9	9	0	9	0	9	0	0	9	0	9	8	9	8	0	0	0	0	0	0	0	0	0	0	0	9	0	9	0	9	0	9	9
rugosa	4	3	0	4	0	7	2	0	6	7	0	7	7	0	7	1	7	0	0	7	0	0	0	7	4	0	0	0	0	0	0	0	0	0	7	0	4	0	0	0	7	0	7	7
saka	1		1	1	1	1	0	1	1	1	0	1	1	0	1	0	1	0	0	1	0	1	0	1	1	0	1	0	0	0	1	0	1	0	1	0	1	1	1	0	1	0	0	0
silvae	1	6	0	0	0	0	0	0	0	7	0	0	7	0	7	0	7	0	0	4	0	0	0	7	6	0	0	0	0	0	1	0	0	0	0	0	5	0	0	0	0	0	6	5
valida	2	5	0	0	0	0	0	0	0	7	0	0	0	0	7	0	7	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	6	3
vini	5	23	0	0	0	0	0	0	0	28	0	0	28	0	28	0	24	0	0	0	0	0	0	28	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	0
zeylanoides	57	48	0	5	0	0	10	0	100	80	0	99	100	7	105	6	105	0	0	35	0	0	0	105	39	0	0	0	0	0	99	0	86	26	104	0	40	0	102	0	0	0	100	10
Cryptococcus albidus var albidus	5	2	0	4	3	7	3	7	7	7	0	7	7	1	7	0	3	7	0	0	7	7	1	7	4	7	7	7	7	7	7	7	0	7	4	7	0	7	7	7	0	7	7	7	0
albidus var albidus	5	2	0	2	2	7	2	7	7	7	0	2	2	2	7	1	3	7	0	3	6	7	2	7	3	0	7	7	7	7	7	7	0	7	2	7	0	7	7	7	0	7	7	7	0
albidus var diffluens	2	1	0	2	2	2	2	2	2	2	0	2	2	2	2	3	1	2	0	2	0	2	2	2	2	0	2	2	0	2	2	0	2	2	2	0	2	2	2	0	2	2	2	0
diffluens		1	0	0	1	1	0	1	1	1	0	1	1	0	1	0	0	1	0	1	1	0	0	1	0	0	0	0	1	1	1	1	1	0	1	0	1	1	1	0	1	0	0	0

CONTINUED

Table 12 continued

Species	No.	Isolates	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
			x			x								x		x									x									x						x				
Cryptococcus gastricus		2	0	0	0	2	0	2	0	0	0	2	2	0	2	0	0	2	0	0	2	2	0	2	0	0	2	0	0	0	0	0	2	0	0	0	2	0	2	0	2	0	2	0
hungaricus	3		0	2	3	3	2	3	3	0	0	3	3	0	3	2	0	3	0	3	2	3	2	3	0	2	3	3	3	3	2	3	3	0	3	0	3	3	3	0	3	0	3	0
laurentii var flavescens	5		0	2	4	5	4	5	5	4	0	5	5	5	5	0	1	5	0	1	5	5	3	5	5	5	5	5	5	5	5	5	5	5	5	1	5	5	5	0	5	0	5	0
laurentii var laurentii	11	10	0	6	15	21	6	21	21	20	21	21	21	4	21	5	7	21	0	11	21	21	9	15	6	21	21	9	20	21	21	21	16	7	11	5	21	21	21	8	21	0	20	13
laurentii var magnus	7		0	6	7	7	7	7	7	7	0	7	7	3	7	3	4	7	0	7	7	7	7	7	7	0	7	7	5	7	7	7	5	5	2	2	7	7	7	0	7	0	6	3
macerans	1		0	0	1	1	1	1	1	1	1	1	1	0	1	0	0	1	0	1	1	1	1	1	1	0	1	0	1	1	0	0	1	1	1	0	1	1	1	0	1	1	1	0
skinneri	2		0	1	2	2	0	2	2	2	0	1	2	2	2	0	0	2	0	1	0	0	0	2	0	0	0	0	0	2	2	2	2	2	2	0	2	0	2	0	2	0	2	0
uniguttulatus	2		0	0	0	2	1	2	0	1	0	2	2	0	2	0	0	2	0	0	0	2	0	1	0	0	2	2	2	0	0	0	1	0	0	0	0	2	1	0	2	0	2	0
Debaryomyces hanseni	65	46	0	29	55	111	35	105	47	98	51	109	108	13	111	16	111	0	4	110	42	111	55	111	55	94	105	109	110	95	111	84	111	65	100	50	111	111	111	28	111	0	100	31
marama	2		0	0	0	2	0	2	2	2	2	2	2	1	2	1	2	0	1	1	1	2	1	2	2	2	2	2	2	0	2	2	2	2	2	2	2	2	2	0	2	0	2	0
Hansenula polymorpha	2		2	0	0	0	0	2	2	2	2	0	2	0	2	0	2	0	0	0	0	2	2	2	2	0	2	2	0	2	2	2	2	2	0	0	2	2	2	2	2	2	0	2
Leucosporidium scopuligenum	1		0	1	0	0	0	1	1	1	0	1	1	0	1	0	1	1	0	0	0	1	0	1	1	0	0	1	0	0	1	1	1	1	0	1	1	1	1	0	1	0	2	0
scottii	8	5	0	1	0	1	0	13	0	13	0	11	9	2	13	0	13	0	0	0	13	13	4	13	7	0	13	13	12	13	1	1	13	6	13	0	0	13	13	2	13	13	13	0
Pichia etchellsii	2		2	2	0	2	2	2	2	2	0	2	2	2	2	2	2	0	0	1	0	2	0	2	2	0	2	2	0	0	2	0	2	2	2	0	2	2	2	0	2	0	0	2
media	3		0	2	0	0	2	2	2	2	2	2	2	0	2	2	2	0	0	0	0	2	2	2	2	0	0	0	0	0	2	0	2	2	2	2	2	0	2	2	2	0	1	0
membranae-faciens	30	8	0	0	0	0	0	0	19	38	0	0	0	7	38	0	30	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	0	36	0	0	0	7	0	22	12
vini var melibiosi	7		0	4	0	2	7	6	7	7	0	7	7	2	7	0	7	0	0	5	0	7	6	7	7	7	7	7	6	0	2	0	7	6	7	7	7	7	7	1	7	0	4	0
vini var vini	19		0	10	0	11	9	19	19	19	0	19	19	8	19	1	19	0	0	15	0	19	17	19	19	0	19	19	0	0	19	0	19	18	19	19	19	19	19	11	19	0	18	0
Rhodotorula glutinis var glutinis	6	5	0	2	6	4	5	11	9	11	0	11	11	2	11	2	11	0	0	10	0	11	3	11	4	0	11	11	11	0	5	5	11	4	10	0	11	11	11	1	11	11	9	7
graminis	4	1	0	0	3	3	0	2	0	5	0	5	5	0	5	0	5	0	0	0	0	0	0	5	2	0	0	0	5	2	3	2	1	2	4	0	5	5	5	0	5	5	4	0
marina	2	1	0	2	3	3	2	3	3	0	0	3	3	2	3	1	3	0	0	3	3	3	0	3	2	0	3	3	3	3	3	3	3	1	3	3	3	3	3	0	3	0	3	0

CONTINUED

Table 12 continued

Species	No.	Isolates	1 *	2	3	4 *	5	6	7	8	9	10	11	12 *	13	14 *	15	16	17	18	19	20	21	22	23 *	24	25	26	27	28	29	30	31	32 *	33	34	35	36	37	38 *	39	40	41	42
Rhodotorula minuta var minuta	5	2	0	0	5	5	0	5	0	5	0	4	5	2	5	0	5	0	0	1	0	0	0	5	0	0	5	0	0	0	5	1	5	2	5	0	5	5	5	0	5	0	3 † 1	1
pallida	3	1	0	0	2	0	0	0	0	0	0	1	4	2	4	0	4	0	0	4	0	0	0	4	3	0	0	0	0	0	0	0	4	0	0	2	4	1	4	0	3	0	0	0
rubra	14	19	0	3	33	33	9	9	30	31	0	32	26	0	33	3	28	0	0	10	0	33	10	25	13	0	33	24	33	0	33	33	30	10	27	0	33	33	33	0	33	0	31	20
Torulopsis candida	9	16	0	2	11	25	5	25	25	25	12	25	25	5	25	12	25	0	0	25	14	25	7	25	8	14	15	25	25	8	25	16	24	8	25	1	25	25	25	0	24	0	22	15
domercqii	1		0	0	0	0	0	0	0	1	0	0	1	0	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0
inconspicua	3		0	0	0	0	2	0	0	0	0	0	0	0	3	0	3	0	0	3	0	0	0	0	2	0	0	0	0	0	0	0	0	3	0	0	3	0	0	0	0	0	1	3
norvegica	2		0	0	0	0	2	2	2	2	0	0	2	0	2	0	2	0	0	2	0	0	0	2	2	0	0	0	0	2	0	0	2	0	0	0	2	0	0	0	2	2	2	0
vanderweltii	3		0	2	0	3	0	0	0	0	0	3	3	2	3	0	3	0	0	0	0	0	0	3	3	0	0	0	0	0	3	0	0	2	3	0	3	0	3	0	3	3	3	0
versatilis	1		1	0	0	0	0	1	0	0	0	1	0	0	1	0	1	0	0	0	1	1	1	1	1	0	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	1	1	0
Trichosporon cutaneum	6	84	0	2	9	85	3	87	73	87	53	88	48	4	90	2	68	86	0	55	90	89	5	79	7	62	68	57	53	75	76	87	73	0	79	0	76	71	64	1	89	0	0	87

* isolates obtained from sulphited and unsulphited sausage (384 in total)

† isolates obtained from the factory surveys (333 in total)

Fermentation

1 Glucose

Assimilation

2 Adonitol
3 D-arabinose
4 L-arabinose
5 Arbutin
6 Cellobiose
7 Citrate
8 Ethanol
9 Erythritol
10 Galactose
11 D-glucitol
12 Gluconate
13 Glucose
14 Glucosamine hydrochloride
15 Glycerol
16 Inositol
17 Inulin
18 Lactate
19 Lactose
20 Maltose
21 Maltotriose
22 Mannitol
23 Mannose
24 Melibiose
25 Melezitose
26 α Methyl glucopyranoside
27 Raffinose
28 Rhamnose
29 Ribitol
30 Ribose
31 Salicin
32 Sorbitol
33 Sorbose
34 Starch
35 Succinate
36 Sucrose
37 Trehalose
38 Xylitol
39 Xylose
40 Pottassium nitrate
41 Growth at 5°C
42 Growth at 37°C

Table 13. The incidence of various yeast species from meat products.

Species	Percentage incidence			
	Sulphited sausage	Unsulphited sausage	Minced beef	Skinless sausages
Bullera alba	0	2.5	0	0
tsugae	0.5	0	0	1.8
Candida albicans	1.1	0	0	0
ciferrii	2.6	0	2.1	1.8
curvata	0.5	0	0	5.6
foliarum	0	0	1.0	0
humicola	2.1	2.5	5.2	1.8
ingens	0.5	0	1.0	0
lipolytica var. *deformans*	1.5	2.5	2.1	1.8
lipolytica var. *lipolytica*	1.1	5.0	2.1	3.6
mesenterica	6.9	5.0	3.1	3.6
ravautii	2.1	0	3.1	1.8

continued

Table 13 continued

Species	Percentage incidence			
	Sulphited sausage	Unsulphited sausage	Minced beef	Skinless sausages
Candida				
rugosa	1.6	0	0	1.8
sake	0	0	0	1.8
silvae	0.5	0	0	0
valida	0.5	0	0	1.8
vini	1.1	2.5	1.0	1.8
zeylanoides 1	14.3	7.5	14.5	23.0
Cryptococcus albidus var. aerius	0.5	2.5	2.1	1.8
albidus var. albidus	1.5	0	0	3.6
albidus var. diffluens	0	0	0	3.6
hungaricus	0	2.5	1.0	1.8
laurentii var. flavescens	1.1	2.5	2.1	0
laurentii var. laurentii	2.6	2.5	4.4	1.8

continued

Table 13 continued

Species	Percentage incidence			
	Sulphited sausage	Unsulphited sausage	Minced beef	Skinless sausages
Cryptococcus laurentii var. magnus	1.1	5.0	3.1	0
macerans	0	0	1.0	0
skinneri-	0.5	0	1.0	0
uniguttulatus	0.5	0	1.0	0
Debaryomyces hansenii 2	21.1	12.5	13.5	9.4
marama	1.1	0	0	0
Hansenula polymorpha	1.1	0	0	0
Leucosporidium capsuligenum	0	0	1.0	0
scottii	2.1	5.0	1.0	1.8
Pichia etchellsii	1.1	0	0	0
media	0.5	2.5	1.0	0
membranaefaciens 3	7.2	7.5	7.3	11.5

continued

Table 13 continued

Species	Percentage incidence			
	Sulphited sausage	Unsulphited sausage	Minced beef	Skinless sausages
Pichia vini var. melibiosi	2.6	0	2.1	1.8
vini var. vini	3.7	7.5	6.25	5.6
Rhodotorula glutinis var. glutinis	2.1	0	2.1	0
graminis	1.1	2.5	1.0	0
marina	0.5	0	1.0	0
minuta var. minuta	0.5	2.5	1.0	0
pallida	1.1	0	1.0	0
rubra 4	2.6	7.5	5.2	1.8
Torulopsis candida 5	2.6	5.0	2.1	0
domericqii	0.5	0	0	0
inconspicua	0.5	2.5	1.0	0
norvegica	0	2.5	1.0	0

continued

Table 13 continued

Species	Percentage incidence			
	Sulphited sausage	Unsulphited sausage	Minced beef	Skinless sausages
vanderwaltii	0.5	0	1.0	1.8
versatilis	0.5	0	0	0
Trichosporon cutaneum	2.6	0	0	1.8
Number of samples	34	7	24	3

* Number of strains studied 384

Number identified with -

1 *C. zeylanoides* 57

2 *D. hansenii* 65

3 *P. membranaefaciens* 30

4 *Rh. rubra* 14

5 *T. candida* 9

guideline set by these authors for reliable test reproducibility. Further affirmation of the reproducibility of the methods used in this study came from the investigation of 16 named isolates obtained from the NCYC. No differences between definitions and laboratory observations were noted.

Fifty one species (Table 13) of yeast were isolated from sausage and minced beef. The majority of the species were assigned to six genera: - Candida, Cryptococcus, Debaryomyces, Pichia, Rhodotorula and Torulopsis. The most frequently isolated species were D. hansenii (59 strains), C. zeylanoides (49 strains) and P. membranaefaciens (30 strains). Although there was no major difference in the range of yeast species isolated from sausage and minced beef, slight variations in the proportions of species isolated appeared to be associated with the presence or absence of sulphite (Table 13). Thus, for example, the incidence of D. hansenii was 10% lower in samples of unsulphited sausage and minced beef as compared with sulphited sausage. A similar but less pronounced trend was observed with C. zeylanoides and C. mesenterica. Conversely the incidence of representatives of the genera Cryptococcus and Rhodotorula was higher in unsulphited sausages and minced beef samples.

Differences (presumed) in composition and places of manufacture of sausages did not appear to influence the nature nor the relative proportions of the yeast species isolated from

sulphited sausage and minced beef. Except for two species, the composition and relative proportions of the yeast flora recovered from skinless sausages were similar to those of the British fresh sausage. The incidence of D. hansenii, the most frequently isolated yeast from sulphited sausages, was 10% lower and that of the most frequently isolated yeast from skinless ones was 10% higher.

(2) Biochemical activities

The assimilatory, fermentative and other activities of yeasts isolated from sausage and minced meat in this study are summarised in Table 14. Only 5% of the yeasts were fermentative [C. albicans (2); C. sake (1); Hansenula polymorpha(2); P. etchellsii (2); T. versatillis (1)] and only ca 10% assimilated nitrate [Cr. albidus var. aerius (5); Cr. albidus var. albidus (5); Cr. albidus var. diffluens (2); Cr. hungaricus (3); Cr. marcerans (1); H. polymorpha (2); Leucosporidium scottii (8); Rh. glutinis (6); Rh. graminis (5); T. domercqii (1); T. norvegica; T. vanderwaltii (3); T. versatilis (1)] as a sole source of nitrogen. A wide range of carbon sources were assimilated to a greater or lesser extent (Table 14). There was no appreciable difference in the assimilatory properties of yeasts isolated from the various samples. Citric acid, galactose, glucose, glycerol, maltose, mannitol, mannose, salicin, sorbitol, sorbose, succinic acid and sucrose were the compounds assimilated most frequently. Only one quarter

of the yeast isolates assimilated starch. Extracellular amylase activity was demonstrated in cell free supernatants of broth cultures of D. hansenii and T. candida and, to a lesser extent, by Cr. albidus var. albidus and P. membranaefaciens but not those of C. lipolytica var. lipolytica and C. zeylanoides following incubation at 4 and 25°C for 168 and 24 h respectively (Table 15). These observations were in accord with those made in the taxonomic study (Table 14) in which this property was demonstrated by assimilation of starch. A strain of D. hansenii was the only isolate tested that produced glucoamylase. It is noteworthy that the temperature of incubation did not appear to influence the nature or extent of amylase activity of any of the isolates.

Approximately 30% of yeasts isolated from all samples were lipolytic as judged by action on Tweens incorporated in an agar medium (Table 16). Tweens 40 and 60 were broken down by all lipolytic isolates. The lipolytic activity was variable among species of Candida, Cryptococcus, Debaryomyces and Torulopsis. Those of the genera Pichia and Rhodotorula did not breakdown Tweens. Consistently lipolytic species were C. lipolytica var. deformans, C. lipolytica var. lipolytica, Cr. albidus var. aerius, Cr. albidus var. albidus, Cr. albidus var. diffluens, Cr. laurentii var. flavescens, Cr. laurentii var. laurentii, Cr. laurentii var. magnus and Cr. skinneri . In agreement with these observations, extracellular lipase activity towards a wide variety of substrates,

including pork and beef fat, was demonstrated in cell free supernatants of broth cultures of C. lipolytica var. lipolytica, C. zeylanoides, Cr.albidus var. albidus, D. hansenii and T. candida but not in those of P. membranaefaciens and Rh.rubra following incubation at 4 and 25°C for 168 and 24 h respectively (Table 17). The most extensive action on this wide range of substrates was exhibited by C. lipolytica var. lipolytica particularly at incubation temperatures of 25°C. The incubation temperature did not appear to influence the lipolytic activities of the isolates tested except for those of D. hansenii in which the most extensive activities took place following incubation at the higher temperature (25°C).

As gelatin hydrolysis proved to be an unsatisfactory indicator of proteolysis (test reproducibility $\geq$ 5%, the guideline set by Sneath and Jones, 1972), the results were discounted. Proteases were not demonstrated by the diazo hyde powder method in the lab lemco or YEPD broth cultures (incubation at 1 or 25°C for 168 and 24 h respectively) of C. curvata, C. lipolytica var. lipolytica, C. zeylanoides, Cr. albidus var. albidus, D. hansenii, P. membranaefaciens, Rh.rubra and T. candida.

Upwards of half of all the yeasts isolated from all samples grew at 37°C (Table 14) this proportion being highest among isolates from skinless sausage. As a large proportion of the yeasts grew, however, at 5°C (incubation for up to 10 days) they can be considered to be psychrotrophic.

Table 14 Percentage of yeasts isolated from meat products which possessed the ability to assimilate or ferment various compounds.*

Attribute	Sulphited Pork sausage	Unsulphited Pork sausage	Minced beef	Skinless sausage
Assimilation of				
Adonitol	27	33	27	-
D-Arabinose	26	35	30	28
L-Arabinose	60	50	48	49
Arbutin	44	63	50	-
Cellobiose	60	57	63	40
Citrate	76	67	64	54
Erythritol	26	22	24	38
Ethanol	60	55	56	62
Galacticol	27	38	36	30
Glucosamine hydrochloride	25	30	17	-
Galactose	75	67	76	70
Glucitol	43	33	32	34
Gluconate	43	43	31	-
Glucose	100	100	100	100
Glycerol	86	75	78	98
Inositol	13	22	22	26
Inulin	1	0	1	0

continued

Table 14 continued

Attribute	Sulphited Pork sausage	Unsulphited Pork sausage	Minced beef	Skinless sausage
Lactate	60	48	57	55
Lactose	15	27	25	20
Maltose	68	65	65	53
Maltotriose	57	65	52	-
Mannitol	72	82	79	96
Mannose	84	91	79	-
Melibiose	21	20	36	26
Melezitose	49	57	46	50
∝Methyl-glycopyranoside	54	55	49	45
Potassium nitrate	9	13	8	11.3
Raffinose	44	50	43	41
Rhamnose	26	33	32	39.6
Ribitol	48	45	44	38
Ribose	27	32	38	41
Salicin	77	77	73	50
Sorbitol	69	75	70	-
Sorbose	83	74	68	90
Starch	25	20	29	17
Succinate	79	77	64	83
Sucrose	66	62	58	50

continued

Table 14 continued

Attribute	Sulphited Pork sausage	Unsulphited Pork sausage	Minced beef	Skinless sausage
Trehalose	85	72	78	79
Xylitol	16	18	17	-
Xylose	65	63	63	53
Fermentation of glucose	4.3	4.7	2.1	3.6
Lipolytic Activities	27	37.5	30	24
Growth at 37°C	50	42.5	46	66
5°C	75	92	89	80
Number of yeast studied	195	40	95	54

Table 15 Extracellular amylase activity of 7 yeast species isolated from sausage.

Species	General Amylase		Glucoamylase	
	4≠	25	4	25
Candida curvata	10*	6	0	0
Candida zeylanoides	0	0	0	0
Cryptococcus albidus var. albidus	18	20	0	0
Debaryomyces hansenii	23	20	12+	16
Pichia membranaefaciens	4	9	0	0
Rhodotorula rubra	0	0	0	0
Torulopsis candida	20	25	0	0

≠ Incubation temperature (°C)

* µg reducing sugar generated $h^{-1}/10^8$ cells

+ µg glucose generated $h^{-1}/10^8$ cells

Table 16 The lipolytic activities of yeasts isolated from meat products with Tweens 20, 40, 60 and 80.

Species	†	Sulphited sausage	Unsulphited sausages	Minced beef	Skinless sausage
Bullera alba	1		2,4,6,8(1)*		
tsugae	2	0(1)			0(1)
Candida albicans	2	2,4,6,8(2)			
ciferrii	8	0(3),4(1),4,6,(1)		0(2)	0(1)
curvata	4	0(1)			0(1),2,4,6,8(2)
foliarum	1			0(1)	
humicola	11	0(4)	0(1)	0(5)	0(1)
ingens	2	0(1)		0(1)	
lipolytica var. deformans	7	2,4,6,8(3)	2,4,6,8(1)	2,4,6,8(2)	2,4,5,8(1)
lipolytica var. lipolytica	8	2,4,6,8(2)	2,4,6,8(2)	2,4,6,8(2)	2,4,6,8(2)
mesenterica	21	0(13),2,4,6,8(1)	0(2)	0(3)	0(2)
ravautii	8	0(2),4,6(1),2,4,6,8(1)		2,4,6,8(3)	2,4,6,8(1)
rugosa	4	0(3)			0(1)

continued

Table 16 continued

Species		Sulphited sausage	Unsulphited sausage	Minced beef	Skinless sausage
Candida	+				
sake	1				0(1)
silvae	1	0(1)			
valida	2	2,4,6,8(1)			2,4,6,8(1)
vini	5	2,4,6,8(2)	2,4,6,8(1)	2,4,6,8(1)	2,4,6,8(1)
zeylanoides	57	0(6),4,6(4),2,4,6,8(18)	0(2),4,6(1)	0(6),2,4,6,8(8)	0(3),4,6(1)2,4,6,8(8)
Cryptococcus albidus var. aerius	5	2,4,6,8(1)	2,4,6,8(1)	2,4,6,8(2)	2,4,6,8(1)
albidus var. albidus	5	2,4,6,8(3)			2,4,6,8(2)
albidus var. diffluens	2				2,4,6,8(2)
hungaricus	3		0(1)	0(1)	0(1)
laurentii var. flavescens	5	2,4,6,8(2)	2,4,6,8(1)	2,4,6,8(2)	
laurentii var. laurentii	11	4,6(1),2,4,6,8(4)	2,4,6,8(1)	2,4,6,8(4)	2,4,6,8(1)
laurentii var. magnus	7	2,4,6,8(2)	4,6(1),2,4,6,8(1)	4,6(1),2,4,6,8(2)	
macerans	1			2,4,6,8(1)	

continued

Table 16 continued

Species		Sulphited sausage	Unsulphited sausage	Minced beef	Skinless sausage
Cryptococcus skinneri	2	4,6(1)		4,6(1)	
uniguttulatus	2	0(1)		0(1)	
Debaryomyces hansenii	65	0(36),4,6(4),2,4,6,8(2)	0(4),4,6(1)	0(13)	0(4),2,4,6,8(1)
marama	2	0(2)			
Hansenula polymorpha	2	0(2)			
Leucosporidium capsuligenum	1			0(1)	
scottii	8	0(2),4,6(1),2,4,6,8(1)	0(2)	0(1)	0(1)
Pichia etchellsii	2	0(2)			
media	3	0(1)	0(1)	0(1)	
membranaefaciens	30	0(14)	0(3)	0(7)	0(6)
vini var. melibiosi	7	0(4),4,6(1)		0(1)	0(1)
vini var. vini	19	0(7)	0(3)	0(6)	0(3)
Rhodotorula glutinis var. glutinis	(6	0(4)		0(2)	

continued

Table 16 continued

Species		Sulphited sausage	Unsulphited sausage	Minced beef	Skinless sausage
Rhodotorula	4				
graminis	4	0(2)	0(1)	0(1)	
marina	2	4,6(1)		2,4,6,8(1)	
minuta var. minuta	3	0(1)	0(1)	0(1)	
pallida	3	0(2)		0(1)	
rubra	14	0(5)	0(3)	0(5)	0(1)
Torulopsis candida	9	2,4,6,8(5)	2,4,6,8(2)	4,6(2)	
domercqii	1	0(1)			
inconspicua	3	4,6(1)	4,6(1)	4,6(1)	
norvegica	2		0(1)	0(1)	
vanderwaltii	3	0(1)		2,4,6,8(1)	0(1)
versatilis	1	0(1)			
Trichosporon cutaneum	6	0(5)			0(1)

continued

Table 16 continued

CODE:- 0 - no lipolysis

2 - Lipolysis of Tween 20

4 - Lipolysis of Tween 40

6 - Lipolysis of Tween 60

8 - Lipolysis of Tween 80

+ - Total number of isolates in parenthesis

* - number of isolates in parenthesis

Table 17 Extracellular lipase activity of stationary phase cultures of 7 yeast species isolated from sausage.

		Pork fat	Beef fat	Olive Oil	Triacetate	Tricaprionate	Trioleate	Tr butyrin
Candida lipolytica var. lipolytica	1	13.1*	13.1	12.5	10.0	15.0	11.5	12.5
	2	9.5	9.5	7.5	7.0	15.0	7.5	12.5
C. zeylanoides	1	1.5	2.0	1.5	1.5	4.0	9.0	3.6
	2	1.0	1.5	1.5	1.5	3.0	7.5	2.5
Cryptococcus albidus var. albidus	1	1.0	2.5	1.0	1.0	5.5	5.0	1.5
	2	1.0	1.5	1.0	1.0	5.0	5.5	1.5
Debaryomyces hansenii	1	3.0	3.5	6.0	5.0	8.0	7.0	4.0
	2	2.5	2.0	3.0	2.5	7.5	6.0	3.5
Pichia membran-aefaciens	1	0	0	2.5	0	1.5	1.5	0
	2	0	0	1.0	0	0	0	0
Rhodotorula rubra	1	0	0	1.5	1.0	0	0	0
	2	0	0	0	0	0	0	0
Torulopsis candida	1	1.5	1.5	2.0	1.5	7.5	4.5	1.5
	2	1.0	1.0	1.0	1.5	7.0	4.0	3.0

* 1 unit = equivalents of NaOH / 10^8 cells 1 Incubation 24 h 25°c 2 Incubation 168 h 4°c

(3) Bismuth sulphite tolerance

As noted previously (pp7-9) free sulphite is assumed to be the most probable form of the preservative in the British fresh sausage. It was considered, therefore, that the ability of an organism to grow on malt extract agar containing 20, 50 or 80% bismuth sulphite agar (lab m) may provide an index of its tolerance of the preservative - the latter assumption was based on extensive experience in the fruit juice industry (R.R. Davenport, pers.comm.). Growth on 20% was scored as 1, on 50% 2 and 80% bismuth sulphite agar 4 and the sum of scores was used as an index of sulphite tolerance. Thus a sum of 1 indicated a low tolerance and 7 a high tolerance. It is evident from Table 18 that, in general, the tolerance of bismuth sulphite was a variable attribute of isolates of the yeast species of either sausage or minced beef origin inferring that the presence of sulphite did not exert a strongly elective action.

Source of the yeast flora in the British fresh sausage - Factory Survey

Once the identity of the yeast flora of the British fresh sausage had been established a further study to determine the source of yeast infection during the manufacture of this commodity in a large factory having its own abattoir was undertaken. The level of contamination of three pigs was followed from the slaughter line to the finished product on two occasions. A total of 333 yeast isolates from this source were characterised in detail (Tables 11 and 12) and identified according to the

Table 18 The Bismuth sulphite tolerance codes of yeasts isolated from sausage and minced beef samples

Species		Sulphited sausages	Unsulphited sausages	Minced beef	Skinless sausages
Bullera alba	*1		1(1) +		
tsugae	2	7(1)			7(1)
Candida albicans	2	3(1),7(1)			
ciferrii	8	3(2),7(3)		3(2)	3(1)
curvata	4	7(1)			3(2),7(1)
foliarum	1			1(1)	
humicola	11	0(1),1(1),3(1),7(1)	7(1)	3(5)	3(1)
ingens	2	0(1)		7(1)	
lipolytica var. deformans	7	1(1),7(2)	7(1)	7(2)	7(1)
lipolytica var. lipolytica	8	3(1),7(1)	7(2)	7(2)	7(2)
mesenterica	21	7(14)	0(1),7(1)	7(3)	7(2)
ravautii	8	0(1),7(3)		3(1),7(2)	7(1)
rugosa	4	0(1),7(2)			7(1)

continued

Table 18 continued

Species		Sulphited sausages	Unsulphited sausages	Minced beef	Skinless sausages
Candida sake	1				3(1)
silvae	1	3(1)			
valida	2	0(1)			3(1)
vini	5	1(2)	7(1)	1(1)	3(1)
zeylanoides	57	0(3),1(3),3(8),7(14)	3(2),7(1)	0(1),1(3),3(4),7(6)	0(1),1(1),3(2),7(8)
Cryptococcus albidus var. aerius	5	7(1)	3(1)	1(1),3(1)	3(1)
albidus var. albidus	5	7(3)			7(2)
albidus var. diffluens	2				3(1),7(1)
hungaricus	3		3(1)	1(1)	1(1)
laurentii var. flavescens	5	0(1),7(1)	0(1)	1(2)	
laurentii var. laurentii	11	0(1),3(2),1(1),7(1)	3(1)	0(1),3(2),7(1)	1(1)
laurentii var. magnus	7	3(1),7(1)	1(1),3(1)	1(1),3(1),7(1)	
macerans	1			0(1)	

continued

Table 18 continued

Species		Sulphited sausages	Unsulphited sausages	Minced beef	Skinless sausages
Cryptococcus skinneri	2	7(1)		3(1)	
uniguttulatus	2	3(1)		3(1)	
Debaryomyces hansenii	65	0(19),1(6),3(7),7(10)	0(1),1(2),7(2)	0(3),1(4),3(3),7(3)	0(1),1(3),7(1)
marama	2	3(1),7(1)			
Hansenula polymorpha	2	1(1),3(1)			
Leucosporidium capsuligenum	1			7(1)	
scottii	8	0(1),1(1),7(2)	7(2)	7(1)	7(1)
Pichia etchellsii	2	0(1),3(1)			
media	3	3(1)	0(1)	3(1)	
membranaefaciens	30	3(5),7(9)	7(3)	3(2),7(5)	3(2),7(4)
vini var. melibiosi	7	0(1),1(1),3(2),7(1)		3(1)	3(1)
vini var. vini	19	1(3),3(1),7(3)	3(1),7(2)	0(2),3(1),7(3)	3(2),7(1)
Rhodotorula glutinis var. glutinis	6	0(2),3(1),7(1)		3(2)	

continued

Table 18 continued

Species		Sulphited sausages	Unsulphited sausages	Minced beef	Skinless sausages
Rhodotorula graminis	4	0(2)	1(1)	3(1)	
marina	2	0(1)		3(1)	
minuta var. minuta	3	3(1)	3(1)	7(1)	
pallida	3	3(1),7(1)		7(1)	
rubra	14	1(1),0(1),3(1),7(2)	3(3)	0(1),1(1),2(1),7(2)	3(1)
Torulopsis candida	9	0(1),1(2),7(2)	3(1),7(1)	3(2)	
domercqii	1	7(1)			
inconspicua	3	7(1)	7(1)	7(1)	
norvegica	2		7(1)	3(1)	
vanderwaltii	3	7(1)		7(1)	7(1)
versatilis	1	7(1)			
Trichosporon cutaneum	6	1(1),3(2),7(2)			0(1)

CODE :- 7 Growth on 80,50 and 20% Bismuth Sulphite agars. 3 Growth on 50 and 20% Bismuth Sulphite agars.
1 Growth on 20% Bismuth Sulphite agar. 0 No growth on Bismuth Sulphite agar.
* Total number of isolates + Number of isolates in parenthesis.

definitions of Lodder (1970)

It is evident from Figure 16 that the numbers of yeasts recovered from swabs of carcasses fell significantly ($p \leq 0.05$) in all areas sampled after washing, dehairing and evisceration. The numbers of yeasts recovered from the swabs of the cut surfaces were significantly ($p \leq 0.05$) higher than those from other areas; this may be attributed to contamination from either the contents of the gut or, more probably, from cutting implements. The numbers of yeasts rose significantly ($p \leq 0.05$) over 24 hours chilled storage to the level of contamination present immediately after slaughter. There was a change, however, in the composition of the yeast flora recovered from the surface swabs of carcasses immediately after slaughter and after chilled storage for 24 hours (Table 19); Tr. cutaneum dominated the flora in the first instance, whereas one comprising the species belonging to the genera Candida, Cryptococcus, Debaryomyces, Pichia, Rhodotorula and Torulopsis obtained in the second. It was notable indeed that representatives of the latter group occurred regularly in sausages included in this survey. Yeast flora of a similar composition was recovered from butchered meats awaiting use in sausage manufacture, in sausage constituents other than red meats (Table 20) and also equipment (Table 21). Indeed, similar levels of yeast contaminants were recovered from all ingredients derived from the carcass, red meats, rinds and fat - the extent of yeasts contamination being negligible in the rusks

Figure 16 The influence of processing on the level of yeast contamination obtained from surface swabs of pig carcasses intended for sausage manufacture.

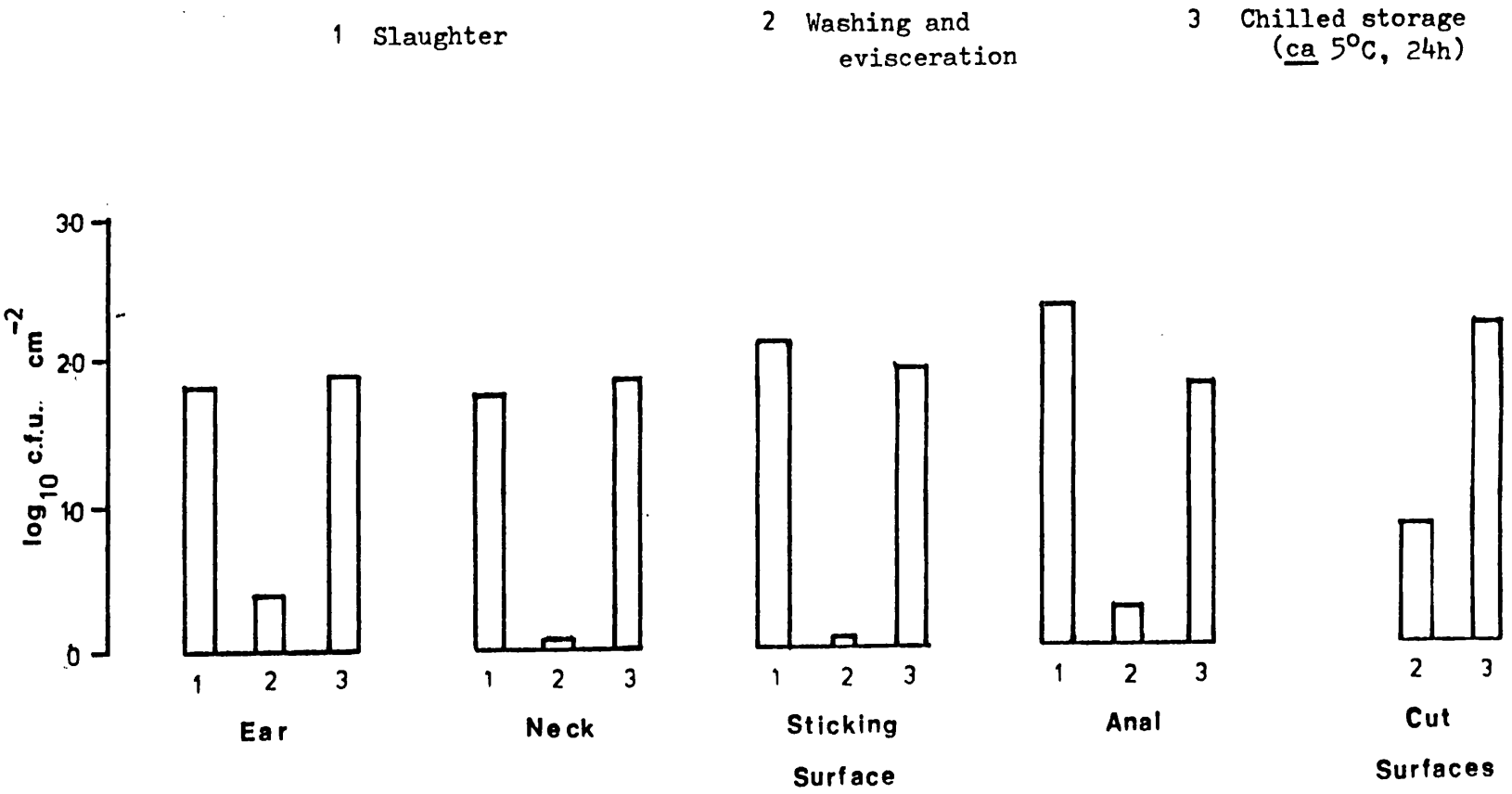


Table 19 The yeasts recovered from carcass swabs.

Stage of production	Ear	Neck	Sticking surface	Anal	Cut surfaces
Slaughter	Tr. cutaneum (18)*	Tr. cutaneum (11)	Tr. cutaneum (12)	Tr. cutaneum (15)	
	D. hansenii (1)	C. vini (1)	C. zeylanoides (12)	D. hansenii (1)	
Yeasts	T. candida (1)	C. zeylanoides (1)	T. candida (1)	C. zeylanoides (1)	
isolated	C. zeylanoides (1)	Rh. rubra (1)			
Evisceration	Tr. cutaneum (1)	Tr. cutaneum (1)		Tr. cutaneum(1)	C. zeylanoides (2)
	D. hansenii (1)	C. vini (1)		C. ciferrii (1)	T. candida (2)
Yeasts	C. curvata (1)			Rh. rubra (1)	C. curvata (1)
isolated					C. ciferrii (1)
					C. silvae (1)
					Cr. gastricus (1)
					Rh. glutinis (1)

continued

Table 19 continued

Stage of production	Ear	Neck	Sticking surface	Anal	Cut surfaces
24 h chill storage	C. zeylanoides (4)	C. zeylanoides (3)	C. zeylanoides (3)	C. zeylanoides (5)	C. vini (4)
	Cr. laurentii (2)	D. hansenii (1)	D. hansenii (2)	C. curvata (3)	D. hansenii (7)
		C. humicola (1)	C. curvata (1)	D. hansenii (2)	T. candida (1)
	D. hansenii (1)	Cr. laurentii (1)	C. humicola (1)	T. candida (2)	P membranaefaciens(1)
	C. ciferrii (1)		C. vini (1)	C. humicola (1)	C. silvae (1)
	C. rugosa (1)	Rh. glutinis (1)	P. membranaefaciens(1)	C. vini (1)	Rh. graminis (1)
	L. scottii (1)		Rh. rubra (1)	P.membranaefaciens (1)	C. curvata (1)
	T. candida (1)		L. scottii (1)		
	Rh. rubra (1)				

* number of isolates

Table 20 The yeasts recovered from sausage constituents.

Sample	Head meat	Belly meat	Shoulder meat	Fat	Rind
Yeasts g^{-1}	1.1×10^3-4.5×10^4	4.15×10^2-3.7×10^4	3.1×10^2-9.7×10^3	7.3×10^2-1.9×10^4	1.1×10^2-4×10^3
Species isolated	C. vini (2)*	C. vini (3)	C. zeylanoides (3)	C. zeylanoides (2)	D. hansenii (2)
	C. curvata (1)	C. curvata (3)	C. vini (3)	P. membranaefaciens (1)	C. silvae (2)
	C. zeylanoides (1)	D. hansenii (2)	D. hansenii (1)	C. lipolytica (2)	C. vini (1)
	C. ciferrii (1)	C. zeylanoides (2)	Cr. albidus (1)	C. vini (1)	C. zeylanoides (1)
	C. lipolytica (1)	T. candida (2)	C. ciferrii (1)	C. silvae (1)	T. Candida (1)
	L. scottii (1)	Cr. laurentii (2)	Rh. rubra (1)	Rh. minuta (1)	Rh. rubra (1)
	T. candida (1)	C. humicola (1)			
	Rh rubra (1)	Rh. marina (1)			
	Rh. glutinis (1)	Rh. pallida (1)			
	Cr. dimennae (1)				

continued

Table 20 continued

Sample	Turkey Wings	Rusk	Seasonings	Mix	Sausage
Yeasts/g	1.5×10^5	9.0×10^1-5×10^2	nr	3.7×10^2-3.3×10^4	6.95×10^2-3.4×10^4
Species isolated	C. valida (3) C. lipolytica (2) P.membranaefaciens (2) D. hansenii (1) C. curvata (1)	D. hansenii (1) C. curvata (1) C. zeylanoides (1)	-	D. hansenii (3) C. zeylanoides (3) C. lipolytica (2) C. ciferrii (2) T. candida (2) C. valida (1) Cr. laurentii (1) Rh. rubra (1)	D. hansenii (4) C. zeylanoides (3) C. lipolytica (2) C. valida (1) T. candida (1) Cr. albidus (1) Cr. laurentii (1) Rh. glutinis (1) Rh. rubra (1)

* number of isolates

nr non recorded

Table 21 The yeasts recovered from lairage and equipment swabs.

Yeasts cm^{-2}	Lairage 3.5×10^1-9.5×10^1	Knives 6.8×10^1-2.5×10^2	Chopping Boards 5.5×10^1-1.7×10^2	Bowl Chopper 3.0×10^1-1.5×10^1	Extruder 1.9×10^1-1.85×10^2
Species Recovered	Trich. cutaneum (16)	C. zeylanoides (3)	C. vini (3)	C. zeylanoides (1)	D. hansenii (1)
	D. hansenii (1)	C. humicola (1)	C. zeylanoides (2)	C. ciferrii (1)	C. ravautii (1)
	C. curvata (1)	C. vini (1)	D. hansenii (1)	C. silvae (1)	L. scottii (1)
	C. vini (1)	C. rugosa (1)	C. curvata (1)	T. candida (1)	C. zeylanoides (1)
	Rh. rubra (1)	Cr. laurentii (1)	Cr. albidus (1)		C. curvata (1)
		D. hansenii (1)	Rh. rubra (1)		P.membranaefaciens(1)
		Rh. minuta (1)			
		Rh. rubra (1)			

* number of isolates

Table 22 Yeast flora in the factory atmosphere.

Sample site	Slaughter	Dehairing	Evisceration	Chill room
mean yeasts m^{-3}	2.4×10^2	5×10^2	1.1×10^2	2×10^1
Yeasts isolated	Tr. cutaneum (5)*	Tr. cutaneum (4)	D. hansenii (2)	C. zeylanoides (2)
	D. hansenii (4)	D. hansenii (3)	C. humicola (2)	C. curvata (1)
	P.membranaefaciens (1)	Rh. rubra (1)		C. rugosa (1)
				Rh. rubra (1)

Sample site	Butchery	Chopping	Manufacturing
mean yeasts/m^3	1.1×10^2	1.25×10^2	1.0×10^2
Yeasts isolated	D. hansenii (1)	Rh. rubra (2)	Cr. laurentii (2)
	C. curvata (1)	D. hansenii (1)	Cr. albidus (1)
	C. zeylanoides (1)		Rh. rubra (1)
	Cr. gastricus (1)		L. scottii (1)
	Rh. glutinis (1)		D. hansenii (1)
	Rh. rubra (2)		

* number of isolates

and seasonings. The level of contamination of the equipment did not vary appreciably throughout the factory.

Conversely the yeast flora of the lairage was dominated by Tr. cutaneum and thus resembled that on the surface of freshly slaughtered pigs. The aerial yeast counts (Table 22), although similar numerically, were found to differ in composition from the Tr. cutaneum dominated flora in the slaughter and dehairing areas of the process to a yeast flora, similar in composition to that associated with the British fresh sausage, in all other sampling locations.

Sulphite binding

Once the source and identity of the yeast flora of the British fresh sausage had been established it was then feasible to address Brown's (1977) conjecture that these organisms were the main cause of sulphite binding.

(1) Sulphite binding in yeast cultures

The sulphite-binding potential of yeasts was investigated with lab lemco broth containing sulphite (500 $\mu g\ ml^{-1}$) The following isolates were used : (1) C. zeylanoides; (2) Cr. albidus var. albidus; (3) D. hansenii; (4) P. membranaefaciens; (5) Rh.rubra and (6) T. candida, and with numbers (3), (4) and (5) NCYC strains were used also.

The presence of sulphite (500 $\mu g\ g^{-1}$) did not appreciably influence the rate of growth, as judged by mean doubling times (Table 23), or the climax populations attained by yeasts other than Rh.rubra (Figure 17 - 22). Its rate of growth was retarded by sulphite. The different isolates did, however, influence markedly the form (i.e. free or bound) in which sulphite occurred (Figure 17 - 22, Table 23) but not the total concentration of sulphite, which did not change appreciably during incubation. This indicated that, as observed in sausages (pp 9-14), oxidative losses were negligible. After an initial lag period (6 h), which coincided with that of the growth curve, the concentration of free sulphite in cultures of C. zeylanoides, D. hansenii, P. membranaefaciens and T. candida cultures diminished rapidly and there was a concominant increase in the amount of bound sulphite. Indeed, it is evident that the rate of binding paralleled the rate of growth of the sulphite binding yeasts until, at the onset of the stationary phase (14 - 16 h incubation), $<50\ \mu g\ ml^{-1}$ of free sulphite remained. Thus, the concentrations of bound sulphite increased progressively during the exponential phase of yeast growth such that 95 - 98% (Table 23) was in this form by the onset of the stationary phase. In contrast, the concentrations of free, bound and total sulphite remained essentially constant in broth cultures of Rh.rubra and Cr. albidus var. albidus (Figure 21, 22). Indeed the proportions were not appreciably different from those of the uninoculated control (Table 23).

Table 23 Mean doubling times of 6 yeast species grown in sulphited* and unsulphited broth cultures and % sulphite bound.

Yeast Species	SO_3^{2-}	Mean doubling time (h)	Bound Sulphite≠
		'	'
Debaryomyces hansenii	+	0.7	98
	-	0.7	-
Pichia membranaefaciens	+	0.68	98
	-	0.68	-
Candida zeylanoides	+	0.6	94
	-	0.65	-
Torulopsis candida	+	0.73	95.5
	-	0.7	-
Cryptococcus albidus	+	0.75	23
	-	0.7	-
Rhodotorula rubra	+	0.9	23
	-	0.65	-
Control	+	-	22

* sulphite (500 $\mu g\ ml^{-1}$)

≠ Determined by the spectrophotometric method of Banks and Board (1982a).

' Esentially similar results were obtained in a repeat experiment and also with strains of P. membranaefaciens, Rh. rubra, and D. hansenii obtained from NCYC.

Incubation temperature 25°C

Figure 17

(a) The influence of sulphite (500 $\mu g\ ml^{-1}$) on the growth of Debaromyces hansenii in lab lemco broths*.

- O unsulphited
- ● sulphited

(b) The fate of free, bound and total sulphite † in cultures of D. hansenii in lab lemco broths*.

- O Free sulphite, yeast
- ● Free sulphite, control
- □ Bound sulphite, yeast
- ■ Bound sulphite, control
- △ Total sulphite, yeast
- ▲ total sulphite, control

* Essentially similar results were obtained from two independent experiments with a strain isolated from sausage and also 1 obtained from NCYC but, in order to conserve space, only 1 graph is presented.

† The concentration of free bound and total sulphite were determined by the method of Banks and Board (1982a).

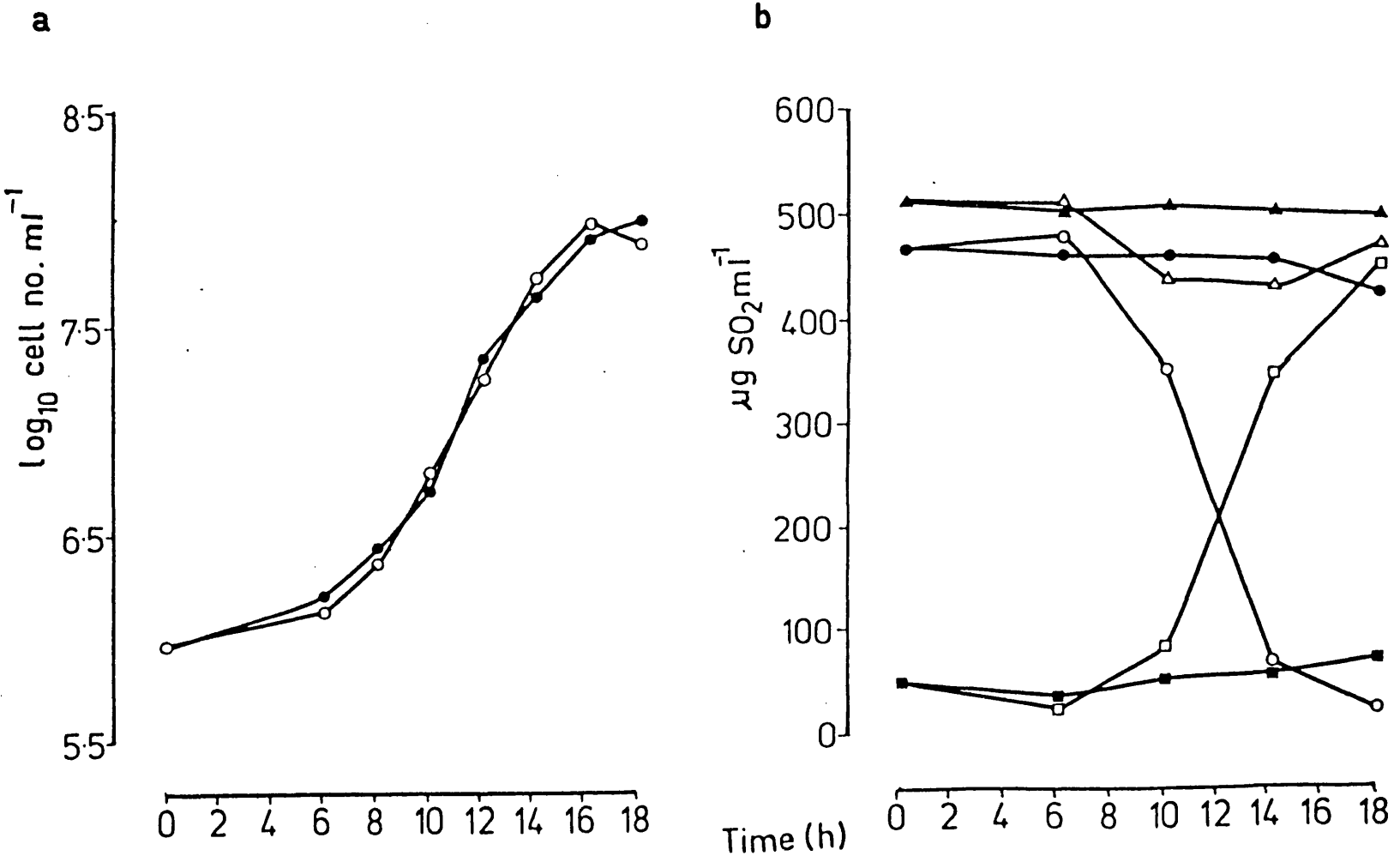

a
b
8·5
7·5
6·5
5·5
log10 cell no. ml-1
600
500
400
300
200
100
0
µg SO2 ml-1
0 2 4 6 8 10 12 14 16 18
Time (h)

Figure 18

(a) The influence of sulphite (500 $\mu g\ ml^{-1}$) on the growth of Pichia membranaefaciens in lab lemco broths*.

- O unsulphited
- ● sulphited

(b) The fate of free, bound and total sulphite + in cultures of P. membranaefaciens in lab lemco broths*.

- O Free sulphite, yeast
- ● Free sulphite, control
- □ Bound sulphite, yeast
- ■ Bound sulphite, control
- △ Total sulphite, yeast
- ▲ Total sulphite, control

* Essentially similar results were obtained from two independent experiments with a strain isolated from sausage and also 1 obtained from NCYC but, in order to conserve space, only 1 graph is presented.

+ The concentration of free bound and total sulphite were determined by the method of Banks and Board (1982a).

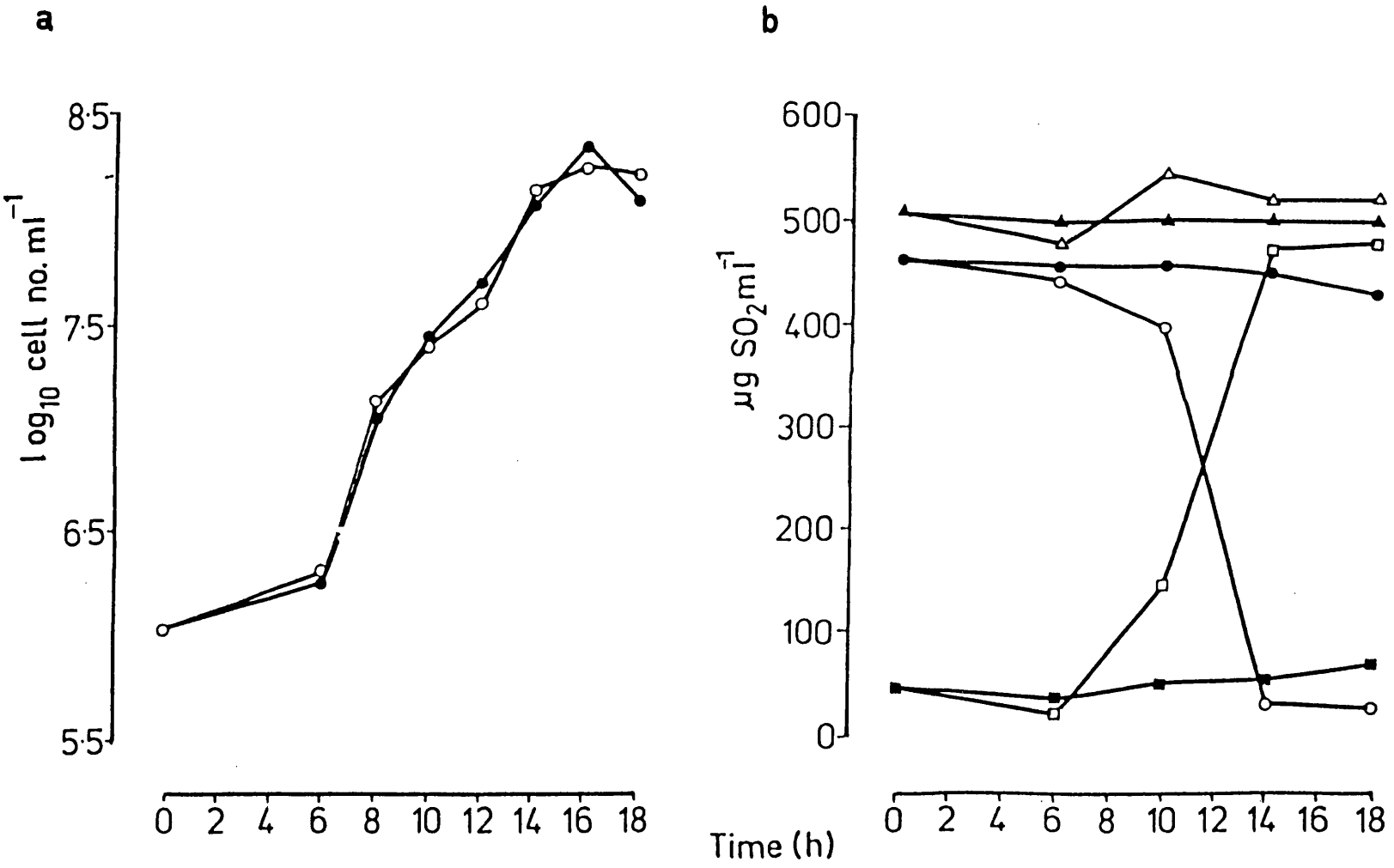

a
b
8·5
7·5
6·5
5·5
log10 cell no. ml-1
600
500
400
300
200
100
0
µg SO2 ml-1
0 2 4 6 8 10 12 14 16 18
Time (h)

Figure 19

(a) The influence of sulphite (500 $\mu g\ ml^{-1}$) on the growth of Candida zeylanoides in lab lemco broths*.

- ○ unsulphited
- ● sulphited

(b) The fate of free, bound and total sulphite† in cultures of C. zeylanoides in lab lemco broths.

- ○ Free sulphite, yeast
- ● Free sulphite, control
- □ Bound sulphite, yeast
- ■ Bound sulphite, control
- △ Total sulphite, yeast
- ▲ Total sulphite, control

* Essentially similar results were obtained from two independent experiments with a strain isolated from sausage but, in order to conserve space, only 1 graph is presented.

† The concentration of free, bound and total sulphite was determined by the method of Banks and Board (1982a).

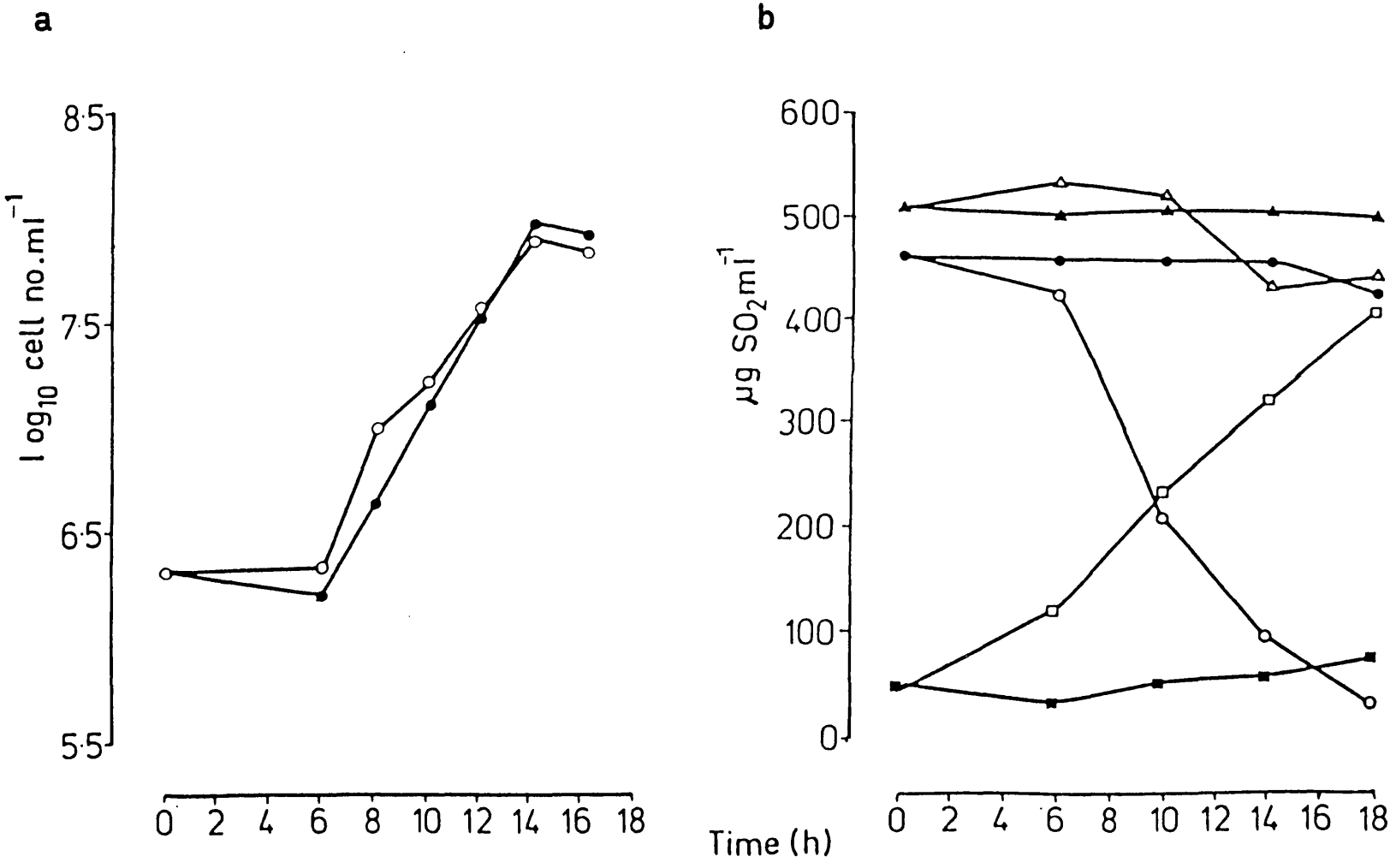

a
b
8·5
7·5
6·5
5·5
log10 cell no.ml-1
600
500
400
300
200
100
0
µg SO2ml-1
0 2 4 6 8 10 12 14 16 18
Time (h)

Figure 20

(a) The influence of sulphite (500 $\mu g\ ml^{-1}$) on the growth of Torulopsis candida in in lab lemco broths*.

- O unsulphited
- ● sulphited

(b) The fate of free, bound and total sulphite† in cultures of T. candida in lab lemco broths.

- O Free sulphite, yeast
- ● Free sulphite, control
- □ Bound sulphite, yeast
- ■ Bound sulphite, control
- △ Total sulphite, yeast
- ▲ Total sulphite, control

* Essentially similar results were obtained from two independent experiments with a strain isolated from sausage but, in order to conserve space, only 1 graph is presented.

† The concentration of free, bound and total sulphite was determined by the method of Banks and Board (1982a).

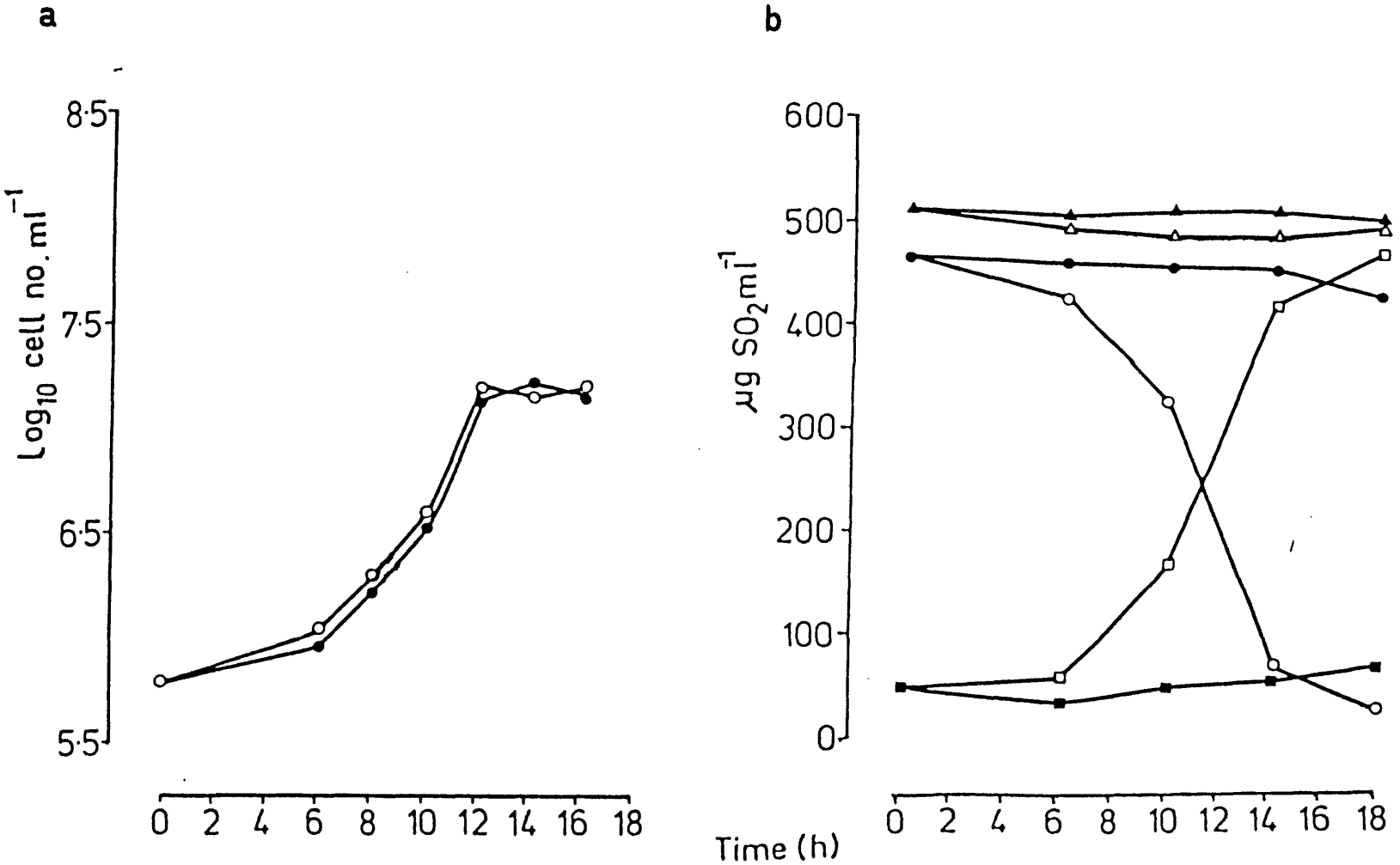

a
b
8·5
7·5
6·5
5·5
Log10 cell no. ml-1
600
500
400
300
200
100
0
µg SO2 ml-1
0 2 4 6 8 10 12 14 16 18
Time (h)

Figure 21

(a) The influence of sulphite (500 μg ml^{-1}) on the growth of Cryptococcus albidus in lab lemco broths*.

- ○ unsulphited
- ● sulphited

(b) The fate of free, bound and total sulphite † in cultures of Cr. albidus in lab lemco broths.

- ○ Free sulphite, yeast
- ● Free sulphite, control
- □ Bound sulphite, yeast
- ■ Bound sulphite, control
- △ Total sulphite, yeast
- ▲ Total sulphite, control

* Essentially similar results were obtained from two independent experiments with a strain isolated from sausage but, in order to conserve space, only 1 graph is presented.

† The concentration of free, bound and total sulphite was determined by the method of Banks and Board (1982a).

a
log10 cell no. ml-1
8·5
7·5
6·5
5·5
0 2 4 6 8 10 12 14 16 18
b
μg SO2 ml-1
600
500
400
300
200
100
0
0 2 4 6 8 10 12 14 16 18
Time (h)

Figure 22

(a) The influence of sulphite (500 μg ml^{-1}) on the growth of Rhodotorula rubra in lab lemco broths*.

- ○ unsulphited
- ● sulphited

(b) The fate of free, bound and total sulphite† in cultures of Rh. rubra in lab lemco broths*.

- ○ Free sulphite, yeast
- ● Free sulphite, control
- □ Bound sulphite, yeast
- ■ Bound sulphite, control
- △ Total sulphite, yeast
- ▲ Total sulphite, control

* Essentially similar results were obtained from two independent experiments with a strain isolated from sausage and also 1 from NCYC but, in order to conserve space, only 1 is presented.

† The concentration of free, bound and total sulphite were determined by the method of Banks and Board (1982a).

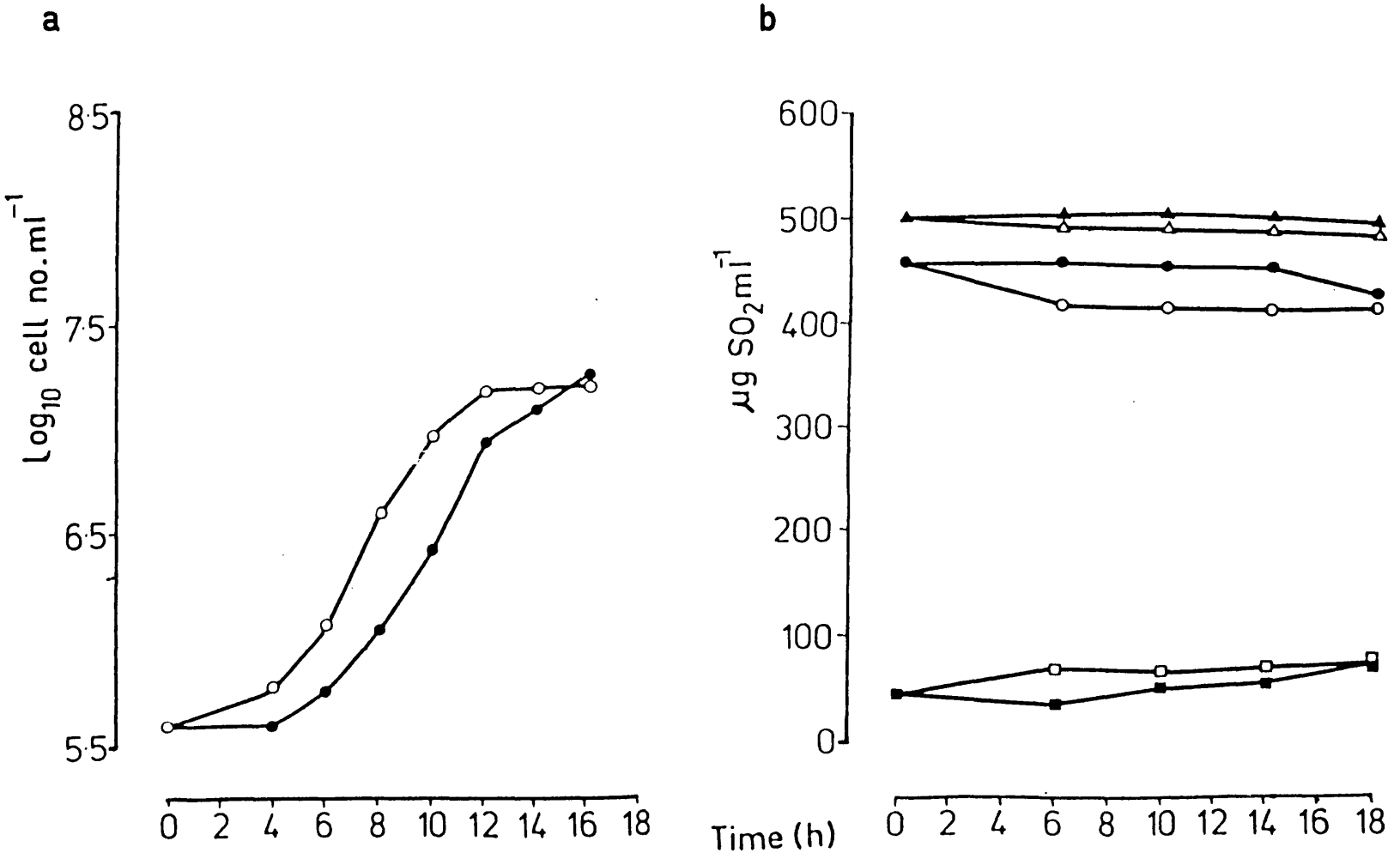

a
b
8·5
7·5
6·5
5·5
Log10 cell no.ml-1
600
500
400
300
200
100
0
µg SO2ml-1
0 2 4 6 8 10 12 14 16 18
Time (h)

Table 24 The pH of stationary phase cultures of 6 yeast species in lab lemco broths containing sulphite*.

Sample	pH
Control	7.0
Debaryomyces hansenii	6.2
Pichia membranaefaciens	6.15
Candida zeylanoides	6.3
Torulopsis candida	6.25
Cryptococcus albidus	6.0
Rhodotorula rubra	6.0

* sulphite (500 $\mu g\ ml^{-1}$)

Table 25 The concentrations ($\mu g\ g^{-1}$) of acetaldehyde, pyruvate and α keto-glutarate in stationary phase broth cultures of 6 yeast species.

Yeast species	SO_3^{2-}*	Acetaldehyde 1	Acetaldehyde 2	Pyruvate 2	α Keto glutarate 2
Debaryomyces hansenii	+	178 (99)	179 (99)	0.67 (<1)	3.0 (<1)
	-	11.7	15.2	7.8	0.6
Pichia membranaefaciens	+	265(128)	259(127)	1.49 (<1)	1.7 (<1)
	-	13.2	14.3	3.1	5.4
Candida zeylanoides	+	138.2(97)	137.1(96)	0.6 (<1)	0.5 (<1)
	-	7.6	6.4	1.8	ND
Torulopsis candida	+	177.2(99)	177.8(96)	4.1 (<1)	1.1 (<1)
	-	7.55	6.35	1.76	ND
Cryptococcus albidus	+	8.8(11)	8.6(11)	4.15 (<1)	1.92 (<1)
	-	6.6	6.0	4.4	3.6
Rhodotorula rubra	+	7.1(6.3)	6.0(4.5)	2.35 (<1)	2.14 (<1)
	-	5.5	5.8	2.26	1.13
Control	+	4.4	4.2	0.49	ND

continued

Table 25 continued

* sulphite (500 µg ml^{-1})

1 Determined using Markham distillation apparatus

2 Enzymic determination (Bernt and Bergmeyer, 1974 a and b)

ND Non detectable

% Sulphite bound in parenthesis (estimated from the binding equilibria of Burroughs and Sparks, 1973)

In all cases the pH of the medium did not change appreciably during incubation (Table 24). The results obtained with strains of D. hansenii, P. membranaefaciens and Rh.rubra from the NCYC were essentially similar to those of isolates of these species from sulphited sausage. Analysis of cell-free medium obtained in the stationary phase of growth of all these species revealed (Table 25) that C. zeylanoides, D. hansenii, P. membranaefaciens and T. candida produced large quantities of acetaldehyde but only small amounts of pyruvate and α keto-glutarate. It was estimated from the results of one experiment, by assuming the binding of acetaldehyde and sulphite to be 1 : 1 (Burroughs and Sparks, 1973; Table 25), that the concentrations of acetaldehyde were sufficient to bind 94 - 98% of the available sulphite. The concentrations of all three compounds (no sulphite) were negligible. Moreover, the concentrations of these compounds in cultures of Cr. albidus var. albidus and Rh. rubra were of the same order as the uninoculated broths.

(2) Sulphite binding in minced pork belly

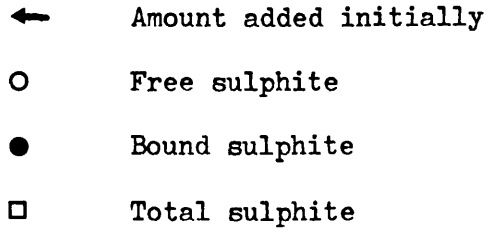
The first part of this section deals with the changes in the adventitious microbial flora and the form and content of sulphite in minced pork. Then the potential of selected members of the microbial association of sausages (pp 31 - 35) to bind sulphite in the following system was examined. Minced pork belly, prepared in a large sausage factory, was supplemented

sequentially with sulphite (500 $\mu g\ g^{-1}$), glucose (20 $mg\ g^{-1}$) or starch (20 $mg\ g^{-1}$) and then seeded ($>10^5$ organisms g^{-1}) with one or other of the following representatives of the association : acetaldehyde producing strain (pp 135-147) of D. hansenii (the most frequently isolated yeast from sausage); Br. thermosphacta; a lactobacillus or a pseudomonad.

The fate of sulphite in minced pork belly

It is evident (Figures 23 - 25) that 170 $\mu g\ g^{-1}$ of sulphite was lost irretrievably during mixing of the minced meat and the supplements. This amount (average 30% +/- 1% n = 3) was of the same order as that lost during normal sausage production (26%; Banks, 1983). The very rapid decline in the concentration of total sulphite during the storage of all the samples of minced pork belly contrasts with the results obtained with sausages both in this study and those of Banks (1983) and also with minced beef (Nychas, 1984). The concentrations of free sulphite decreased rapidly during storage at 1 and 15°C, until 50 $\mu g\ g^{-1}$ remained after 48 hours. The changes thereafter were negligible. The increase in concentration of bound sulphite to ca 280 and 150 $\mu g\ g^{-1}$ in the first 24 h storage at 15 and 1°C respectively was followed by a loss until ca 50 $\mu g\ g^{-1}$ were present after 48 h incubation. Little change occurred thereafter. It is noteworthy that after 24 h storage at 1 and 15°C the concentrations of bound sulphite in samples supplemented with glucose were 150 and 80 µg higher

Figure 23 The concentration of free, bound and total sulphite in minced pork belly stored at (a) 1 and (b) 15°C.



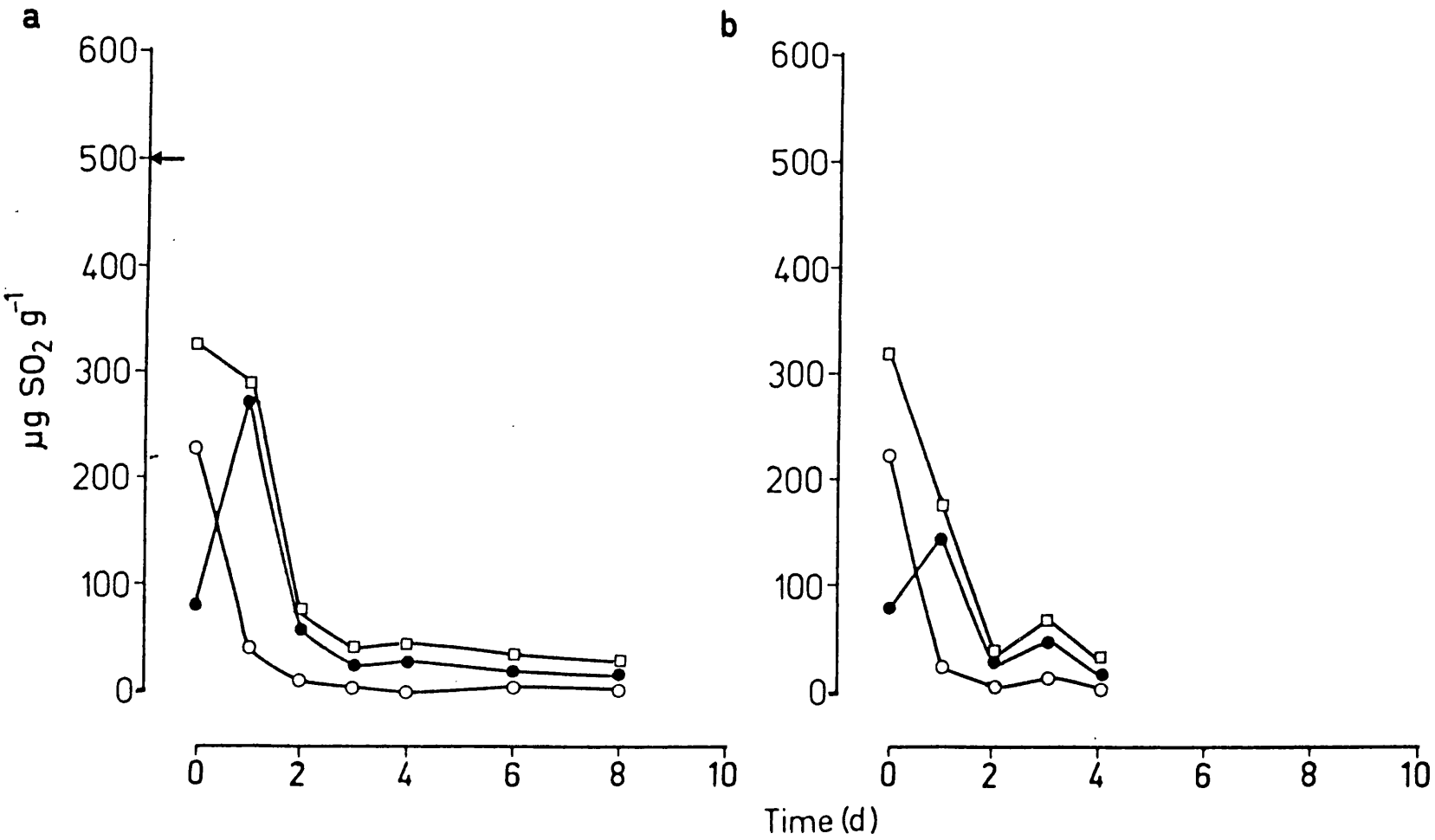
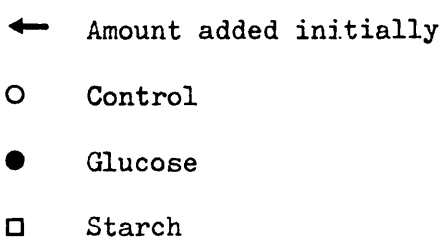

a
b
600
500
400
300
200
100
0
µg SO_2 g^{-1}
0
2
4
6
8
10
Time (d)

Figure 24 Influence of glucose (2% w/w) and starch (2% w/w) on the fate of (a) total, (b) free and (c) bound sulphite in minced pork belly during storage at 1°C.



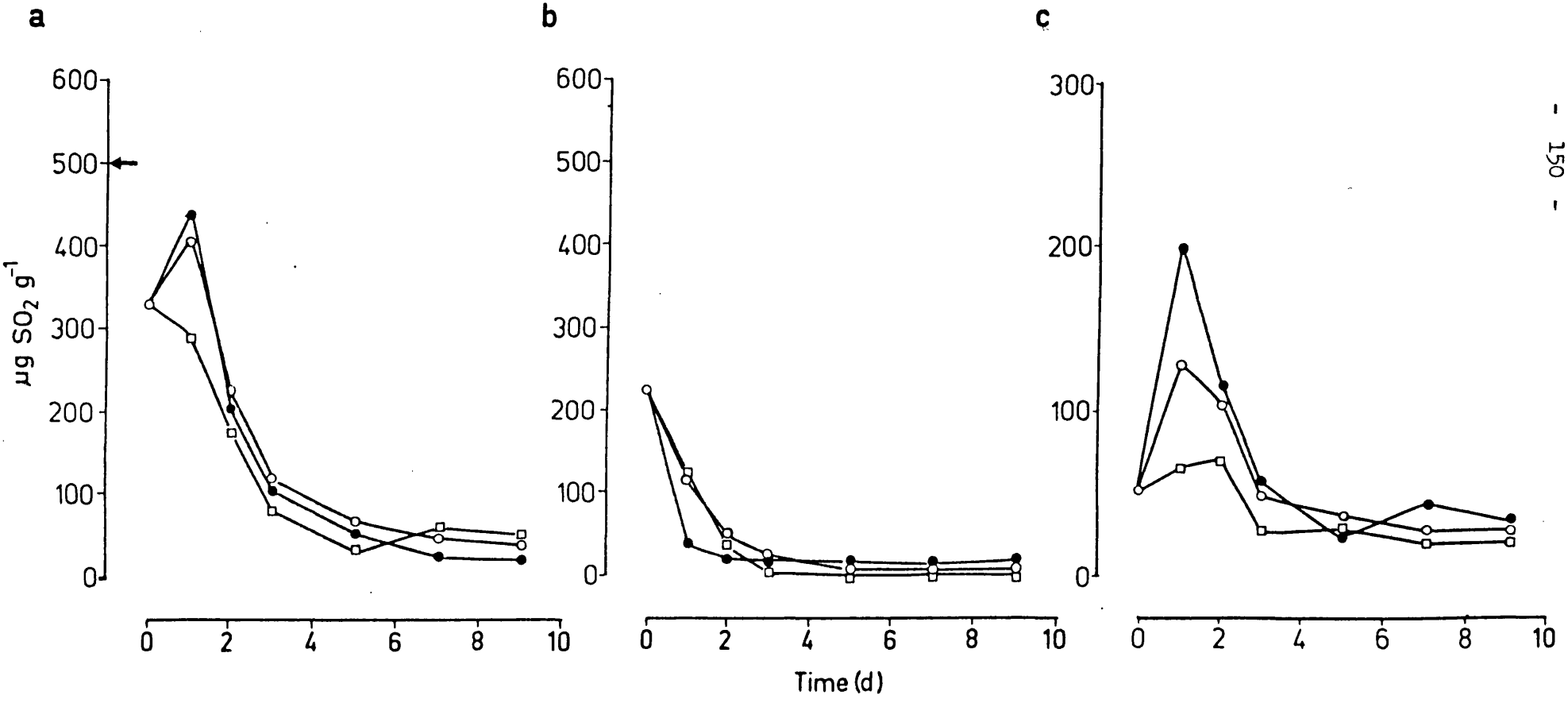
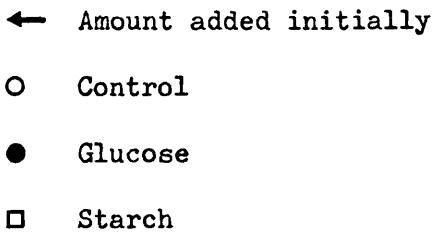
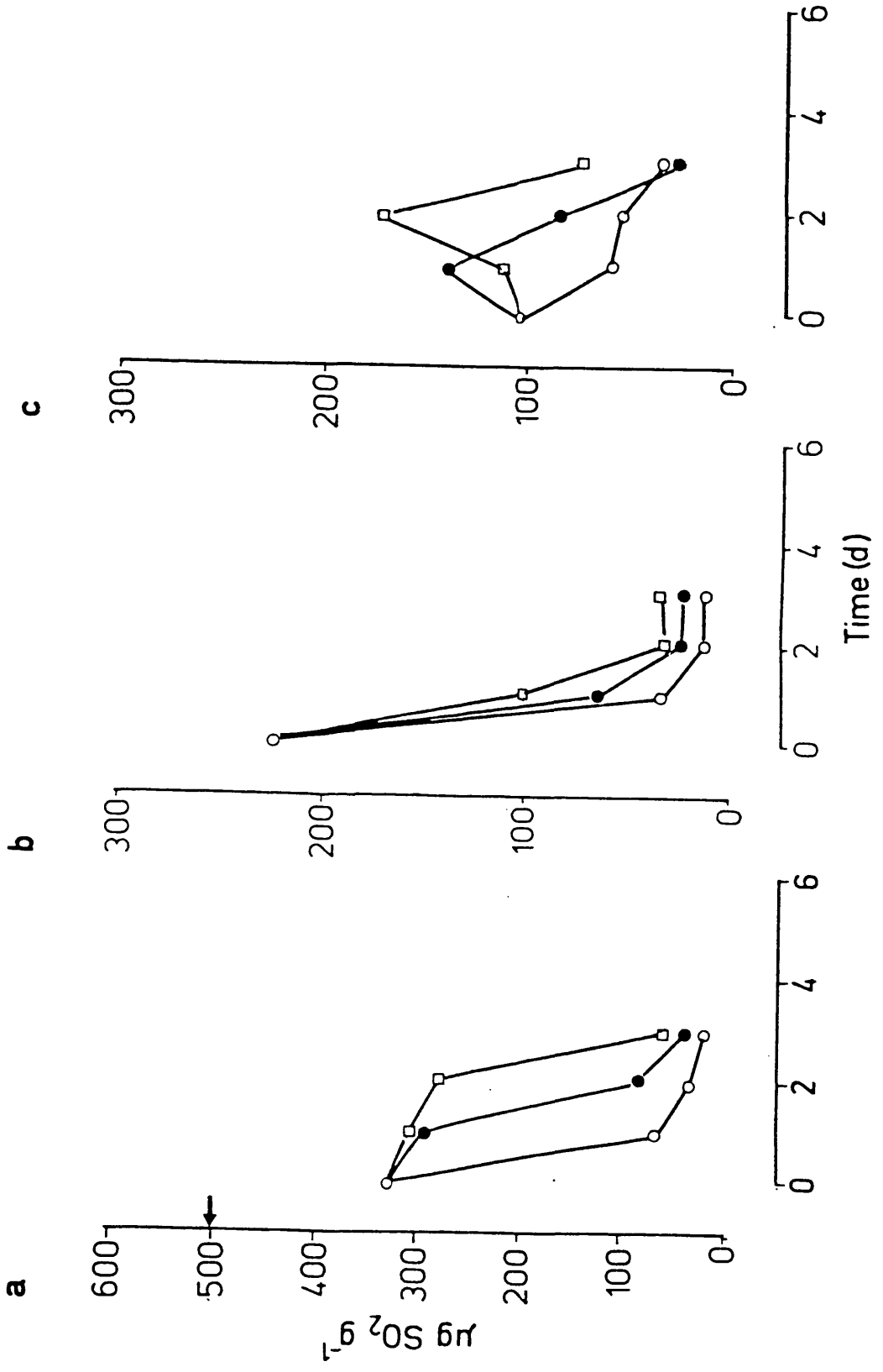

a
b
c
600
500
400
300
200
100
0
300
200
100
0
µg SO2 g-1
0
2
4
6
8
10
Time (d)

Figure 25 Influence of glucose (2% w/w) and starch (2% w/w) on the fate of (a) total, (b) free and (c) bound sulphite in minced pork belly during storage at 15°C.




a
b
c
µg SO2 g-1
600
500
400
300
200
100
0
0
2
4
6
Time (d)

than those of the control (unsupplemented) samples (Figure 23 - 25). In contrast, the concentration of bound sulphite in starch supplemented samples were only appreciably greater than the control (120 $\mu g\ g^{-1}$) in samples after storage for 48 h at 15°C. The subsequent loss of bound sulphite occurred when the concentration of free and total sulphite had decreased below a level of 50 $\mu g\ g^{-1}$. The maximum concentrations of bound sulphite were of the same order as those found in sausages (225 - 480 $\mu g\ g^{-1}$).

The development of the microbial flora

Although sulphite in minced pork did not have an appreciable influence on the size of the climax populations of yeasts, towards the end of storage (Figure 26) it appeared to favour their growth rates as judged by mean doubling times (Tables 26 and 27). In contrast sulphite appeared to retard the rate of growth of Br. thermosphacta (Figure 27, Tables 26 and 27), the numerically dominant organism of minced pork, and lactobacilli also (Figure 28, Tables 26 and 27). The size of the climax populations of the latter appeared to be restricted by sulphite particularly at the higher storage temperature. The rate of growth of pseudomonads (Figure 29, Table 26) and more particularly the enterobacteria (Figure 30, Table 26), were retarded by sulphite. Indeed, the climax populations of the latter were severely curtailed. The curtailment of the size of the climax populations of pseudomonads was smaller than that observed in sausages

Figure 26

(a) The influence of the temperature of storage and sulphite (500 $\mu g\ g^{-1}$) on the growth of yeasts in minced pork belly.

- ○ unsulphited, 1°C
- ● sulphited, 1°C
- □ unsulphited, 15°C
- ■ sulphited, 15°C

(b) The influence of glucose (2% w/w) and starch (2% w/w) on the growth of yeasts in sulphited (500 $\mu g\ g^{-1}$) minced pork belly.

- ○ Control, 1°C
- ● Control, 15°C
- □ Glucose, 1°C
- ■ Glucose, 15°C
- △ Starch, 1°C
- ▲ Starch, 15°C

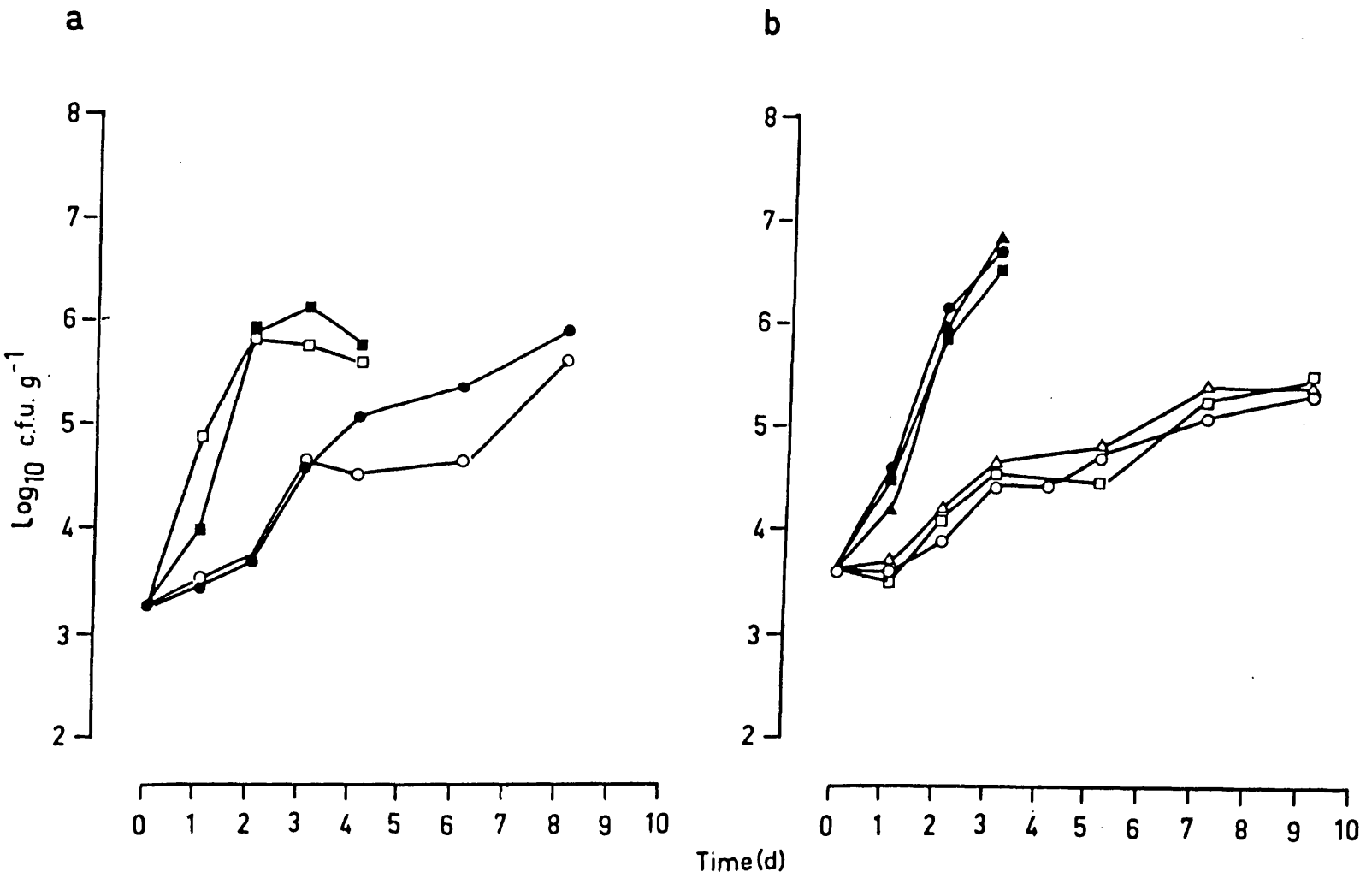

a
b
8
7
6
5
4
3
2
Log_{10} c.f.u. g^{-1}
0 1 2 3 4 5 6 7 8 9 10
Time (d)

Figure 27

(a) The influence of the temperature of storage and sulphite (500 μg g^{-1}) on the growth of Brochothrix thermosphacta in minced pork belly.

- ○ unsulphited, 1°C
- ● sulphited, 1°C
- □ unsulphited, 15°C
- ■ sulphited, 15°C

(b) The influence of glucose (2% w/w) and starch (2% w/w) on the growth of Br. thermosphacta in sulphited (500 μg g^{-1}) minced pork belly.

- ○ Control, 1°C
- ● Control, 15°C
- □ Glucose, 1°C
- ■ Glucose, 15°C
- △ Starch, 1°C
- ▲ Starch, 15°C

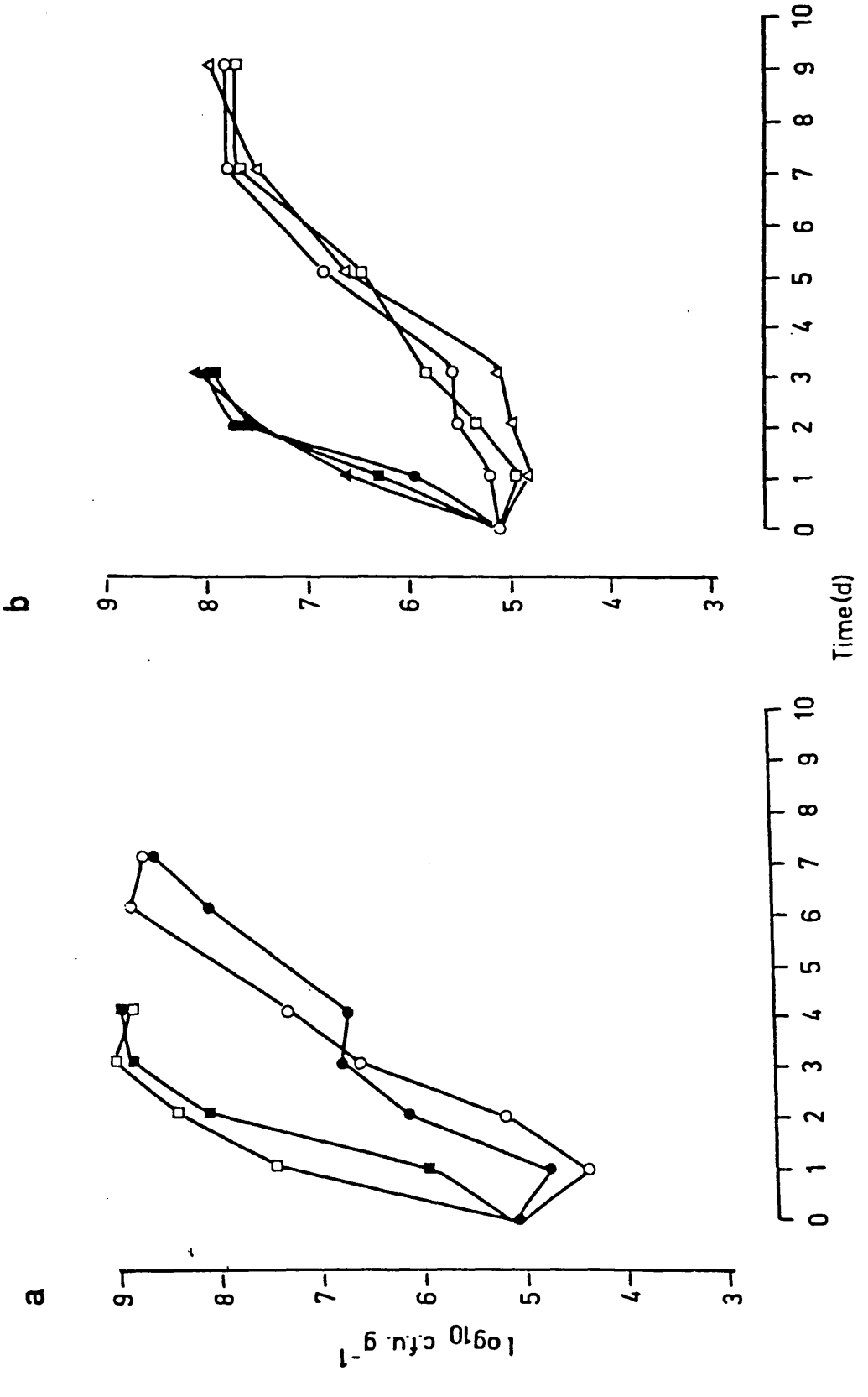

a
b
log10 c.f.u. g-1
9
8
7
6
5
4
3
0 1 2 3 4 5 6 7 8 9 10
Time(d)

Figure 28

(a) The influence of the temperature of storage and sulphite (500 $\mu g\ g^{-1}$) on the growth of lactobacilli in minced pork belly.

- ○ unsulphited, 1°C
- ● sulphited, 1°C
- □ unsulphited, 15°C
- ■ sulphited, 15°C

(b) The influence of glucose (2% w/w) and starch (2% w/w) on the growth of lactobacilli in sulphited (500 $\mu g\ g^{-1}$) minced pork belly.

- ○ Control, 1°C
- ● Control, 15°C
- □ Glucose, 1°C
- ■ Glucose, 15°C
- △ Starch, 1°C
- ▲ Starch, 15°C

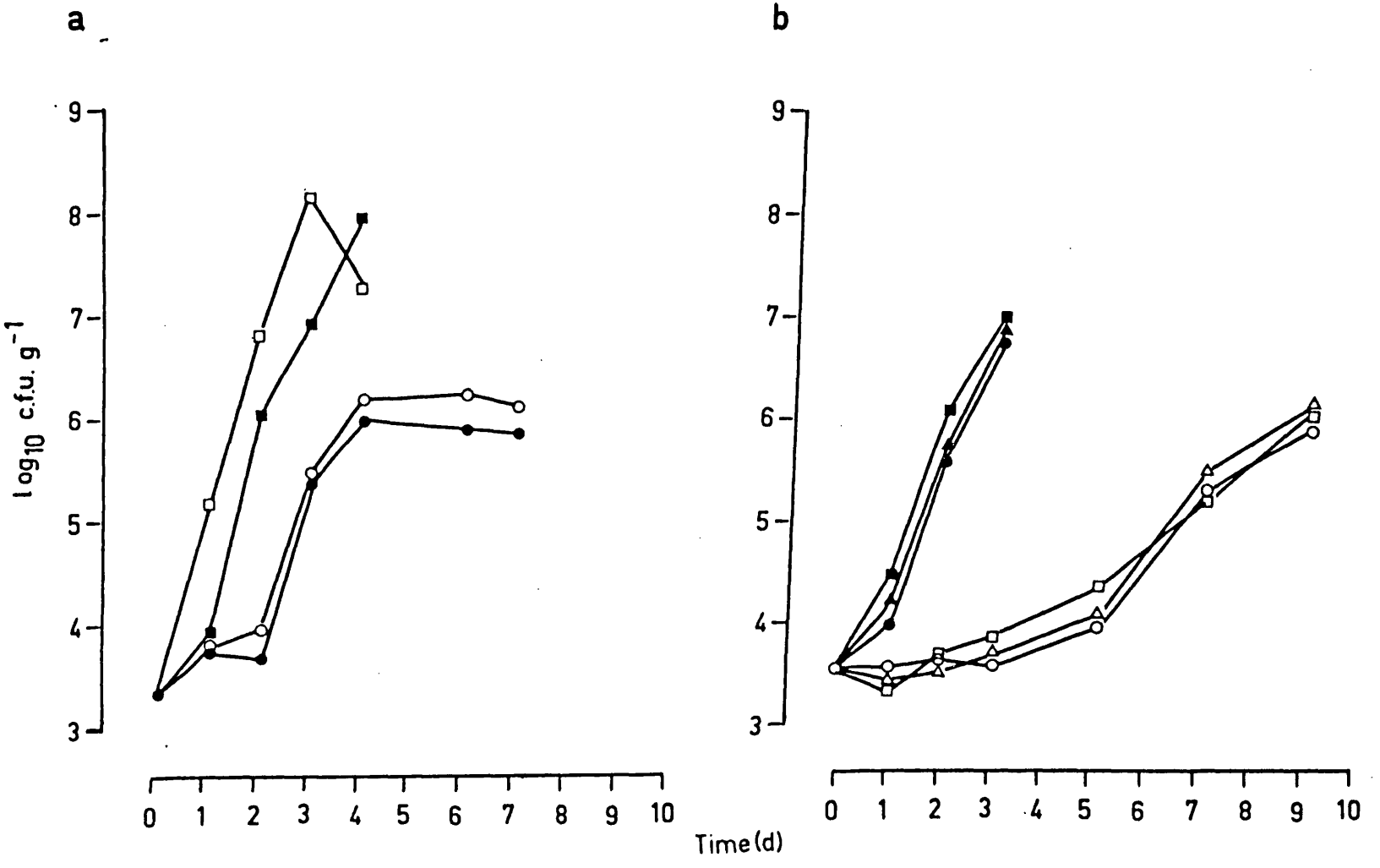

a
b
log10 c.f.u. g-1
9
8
7
6
5
4
3
0 1 2 3 4 5 6 7 8 9 10
Time (d)

Figure 29

(a) The influence of the temperature of storage and sulphite (500 $\mu g\ g^{-1}$) on the growth of Pseudomonads in minced pork belly.

- ○ unsulphited, 1°C
- ● sulphited, 1°C
- □ unsulphited, 15°C
- ■ sulphited, 15°C

(b) The influence of glucose (2% w/w) and starch (2% w/w) on the growth of Pseudomonads in sulphited (500 $\mu g\ g^{-1}$) minced pork belly.

- ○ Control, 1°C
- ● Control, 15°C
- □ Glucose, 1°C
- ■ Glucose, 15°C
- △ Starch, 1°C
- ▲ Starch, 15°C

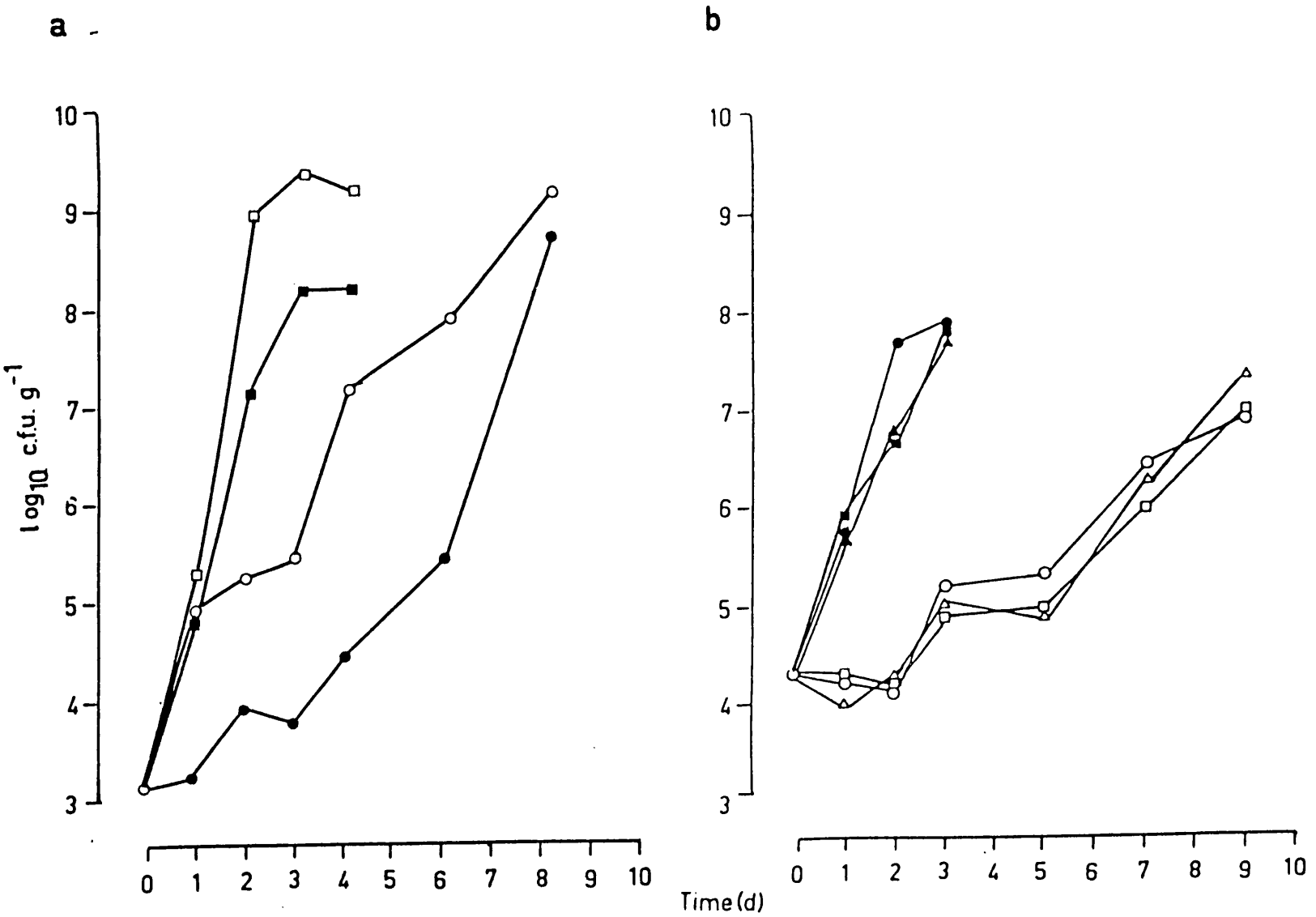

a
b
10
9
8
7
6
5
4
3
log10 c.f.u. g-1
0 1 2 3 4 5 6 7 8 9 10
Time (d)

Figure 30

(a) The influence of the temperature of storage and sulphite (500 $\mu g\ g^{-1}$) on the growth of Enterobacteria in minced pork belly.

- ○ unsulphited, 1°C
- ● sulphited, 1°C
- □ unsulphited, 15°C
- ■ sulphited, 15°C

(b) The influence of glucose (2% w/w) and starch (2% w/w) on the growth of Enterobacteria in sulphited (500 $\mu g\ g^{-1}$) minced pork belly.

- ○ Control, 1°C
- ● Control, 15°C
- □ Glucose, 1°C
- ■ Glucose, 15°C
- △ Starch, 1°C
- ▲ Starch, 15°C

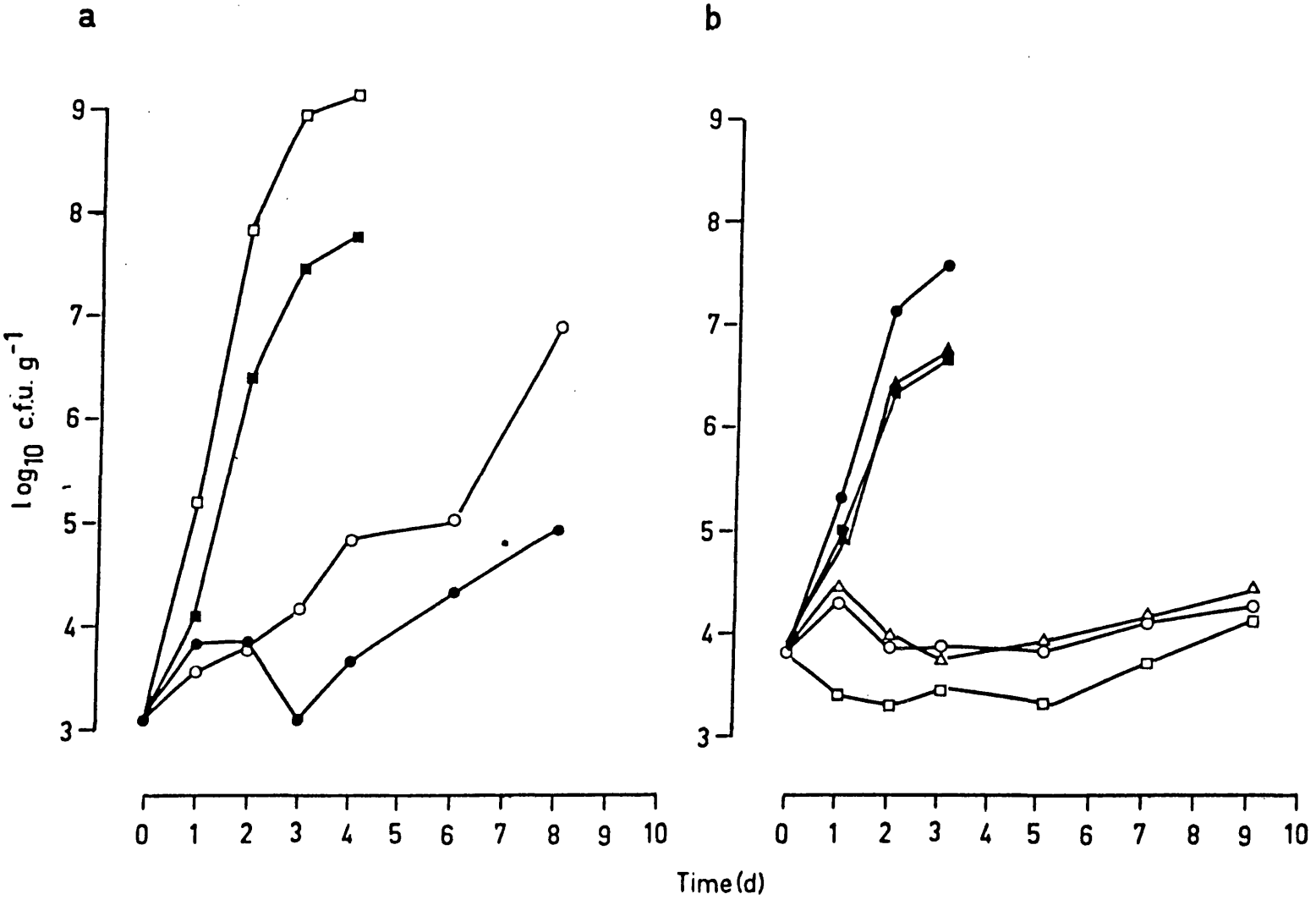

a
b
log10 c.f.u. g-1
9
8
7
6
5
4
3
0 1 2 3 4 5 6 7 8 9 10
Time (d)

Table 26 Influence of sulphite[+] on the mean doubling time (MDT) and climax populations (CP) of yeasts, Brochothrix thermosphacta, lactobacilli, pseudomonads and enterobacteria in pork belly mince.

Organism	* (°C)	MDT (h)		CP ($\log_{10}$ cfug^{-1})	
		-	+	-	+
Yeast	1	24	20	5.6	6.0
	15	7	5	5.8	6.0
Br. thermosphacta	1	7.5	12	8.8	8.4
	15	4	16	8.8	8.6
Lactobacilli	1	11	13	6.2	5.8
	15	4	6.5	8.4	7.8
Pseudomonads	1	9.5	18.5	9.1	8.4
	15	2.5	4.5	9.2	8.4
Enterobacteria	1	15	34	7.6	6.5
	15	3	12	4.5	9.5

* storage temperature

\- Unsulphited

\+ Sulphite included 500 µg g^{-1}

Table 27 Influence of glucose, starch and temperature on the mean doubling time (MDT) and climax populations (CP) of yeasts, Brochothrix thermosphacta, lactobacilli, pseudomonads, and enterobacteria in sulphited pork belly mince (500 $\mu g\ g^{-1}$).

Organism	* (°C)	MDT (h)			CP (log_{10} cfu g^{-1})		
		C	G	S	C	G	S
Yeast	1	38.3	34.3	36.5	5.3	5.4	5.3
	15	7.0	7.5	6.5	6.6	6.6	6.8
Br. thermosphacta	1	24.0	26.0	22.0	7.8	7.7	7.9
	15	5.4	5.7	5.0	7.9	7.7	7.92
Lactobacilli	1	27.0	26.0	25.0	5.9	6.0	6.1
	15	6.7	6.2	6.4	6.7	6.9	6.8
Pseudomonads	1	21.6	24.5	25.0	7.2	6.8	6.7
	15	6.45	6.45	6.45	7.8	7.8	7.7
Enterobacteria	1	144.0	160.0	139.0	4.2	4.0	4.3
	15	40.0	5.66	6.0	7.4	6.5	6.6

KEY
* Storage temperature
C Control sample
G Glucose included 20 mg g^{-1}
S Starch included 20 mg g^{-1}

(0.6 - 0.8 and 2.0 - 2.0 log units g^{-1} respectively). No appreciable differences were observed between the rate and extent of growth of these organisms in sulphited samples supplemented with either glucose or starch (Figures 26 - 30, Table 27). In general, the influence of sulphite on the rate and extent of growth of the microbial flora in supplemented minced pork were in close agreement with those noted with sausages (pp 76 - 92 and Banks, 1983).

pH

There was little change initially in the pH of minced pork belly containing sulphite during storage at 1 or 15°C or in unsulphited material stored at 1°C (Figure 31). Moreover supplementation of sulphited samples with either glucose or starch did not influence appreciably the pH of the samples during storage at either temperature. An acid drift of ca 0.5 units occurred in unsulphited samples stored at 15°. These observations are in accord with those obtained with sausages (pp 92-93). At 1°C, there was a slight alkaline drift at the end of storage of sulphited and unsulphited samples.

The fate of glucose

The concentration of glucose in pork belly mince (Figure 32) diminished with storage particularly at 15°C. The addition of sulphite, however, appeared to exert a sparing effect on the

Figure 31

(a) The influence of the temperature of storage and sulphite (500 $\mu g\ g^{-1}$) on the pH of minced pork belly homogenates.

- ○ unsulphited, 1°C
- ● sulphited, 1°C
- □ unsulphited, 15°C
- ■ sulphited, 15°C

(b) The influence of glucose (2% w/w) and starch (2% w/w) on the pH of sulphited pork belly mince homogenates.

- ○ Control, 1°C
- ● Control, 15°C
- □ Glucose, 1°C
- ■ Glucose 15°C
- △ Starch, 1°C
- ▲ Starch, 15°C

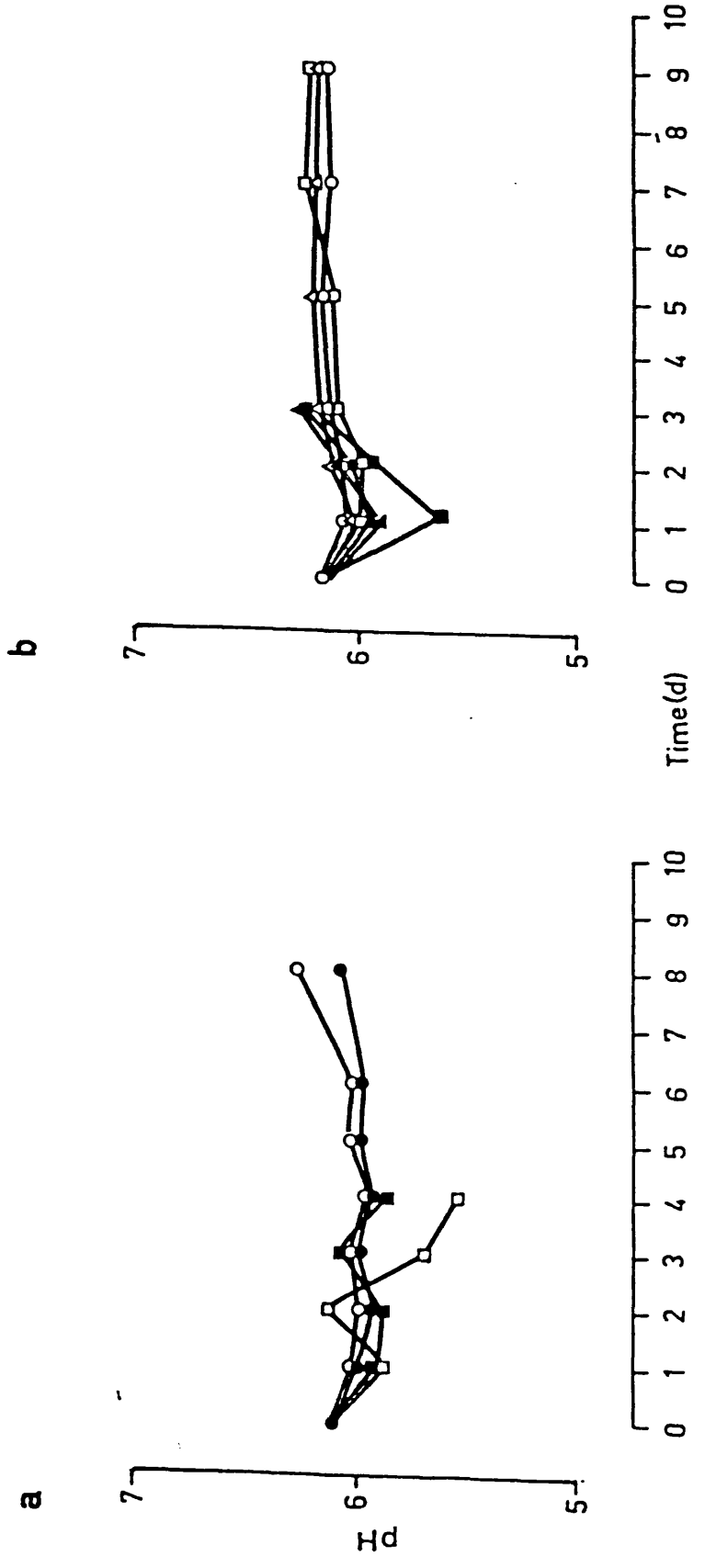
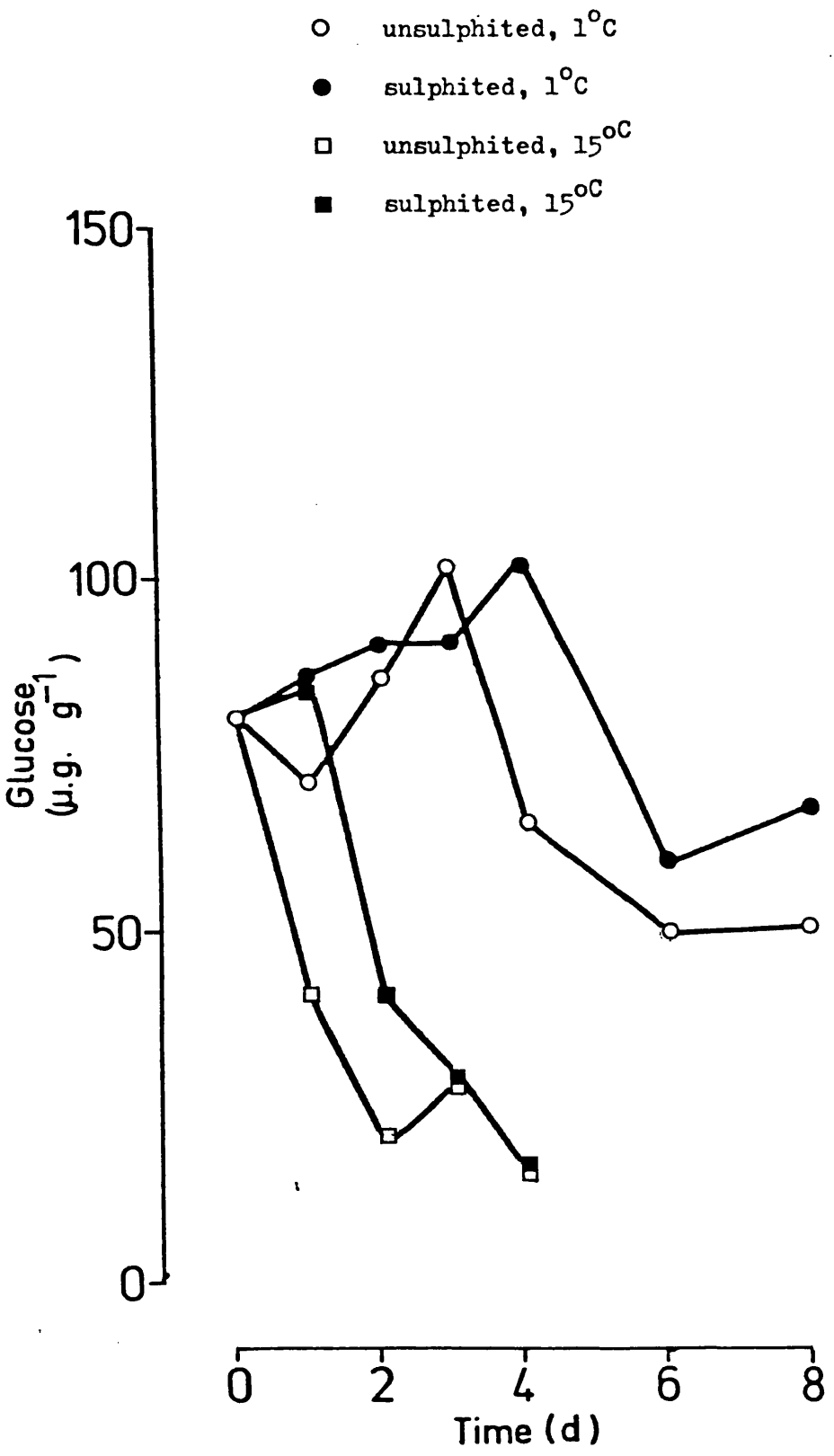

a
b
pH
7
6
5
0 1 2 3 4 5 6 7 8 9 10
Time(d)

Figure 32 The influence of the temperature of storage and sulphite (500 $\mu g\ g^{-1}$) on the concentration of glucose in minced pork belly

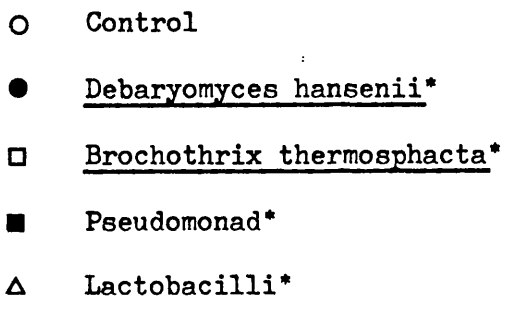


concentration of this sugar at both storage temperatures. This has been demonstrated with sulphited minced beef also (Nychas, 1984).

The development of the microbial flora in inoculated mince

The rate and extent of growth of D. hansenii, Br. thermosphacta, a lactobacillus sp. and pseudomonas sp. (the last mentioned during storage at 15°C only) in unsulphited minced pork were such that their populations maintained numerical dominance over the corresponding adventitious contaminants of the minced meat control (Figure 33 - 36, Table 28). The rate of growth of the yeast was not appreciably influenced by the size of the inoculum (Figure 38). The rate and extent of growth of the resident populations of Br. thermosphacta and pseudomonads were not appreciably influenced by any of the treatments (Figure 34, 36, Table 28). It should be noted, however, that the climax populations attained by enterobacteria in samples seeded with a lactobacillius, Br. thermosphacta and a pseudomonad were smaller than those of the control and yeast-seeded samples (Figure 37, Table 28). In contrast, the rate of growth and the size of the climax populations attained by the yeast and lactobacilli appeared to be enhanced in samples seeded with a lactobacilli or D. hansenii respectively at both storage temperatures (Figure 33, 35, Table 28).

Figure 33 The influence of inoculating sulphited (500 μg g^{-1}) pork belly mince with 4 microbial contaminants* on the rate of growth of yeasts during storage at (a) 1° and (b) 15°C.



* Initial concentration ▷ 10^5 organisms g^{-1}

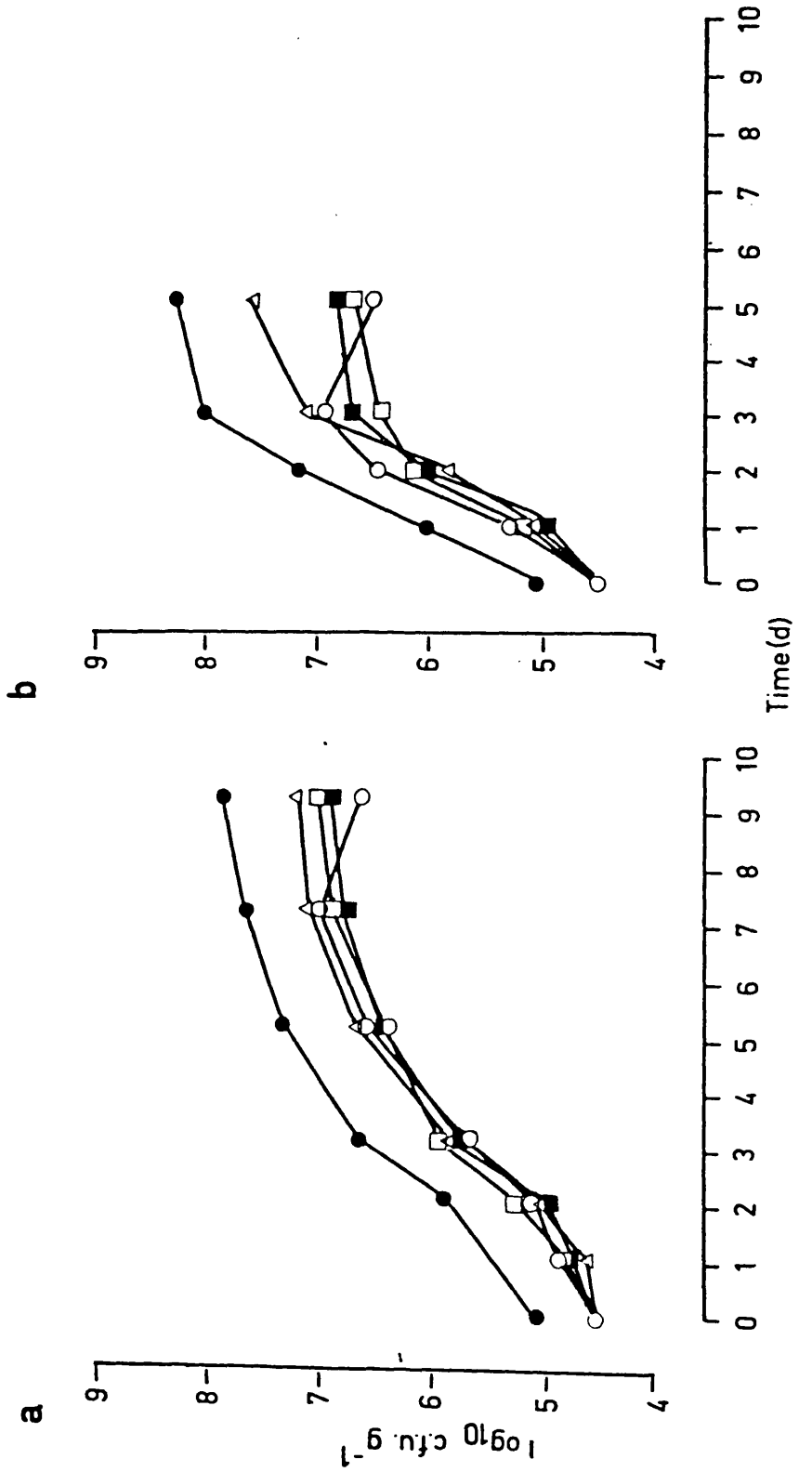
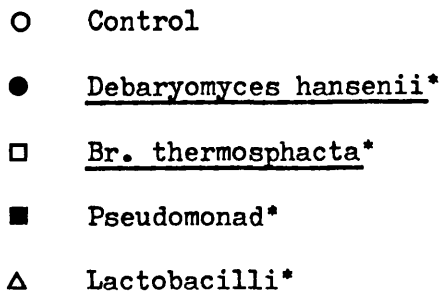

a
b
log10 c.f.u. g-1
9
8
7
6
5
4
0 1 2 3 4 5 6 7 8 9 10
Time(d)

Figure 34 The influence of inoculating sulphited (500 $\mu g\ g^{-1}$) pork belly mince with 4 microbial contaminants* on the rate of growth of Brochothrix thermosphacta during storage at (a) 1 and (b) 15°C.



* Initial concentration ▷ 10^5 organisms g^{-1}

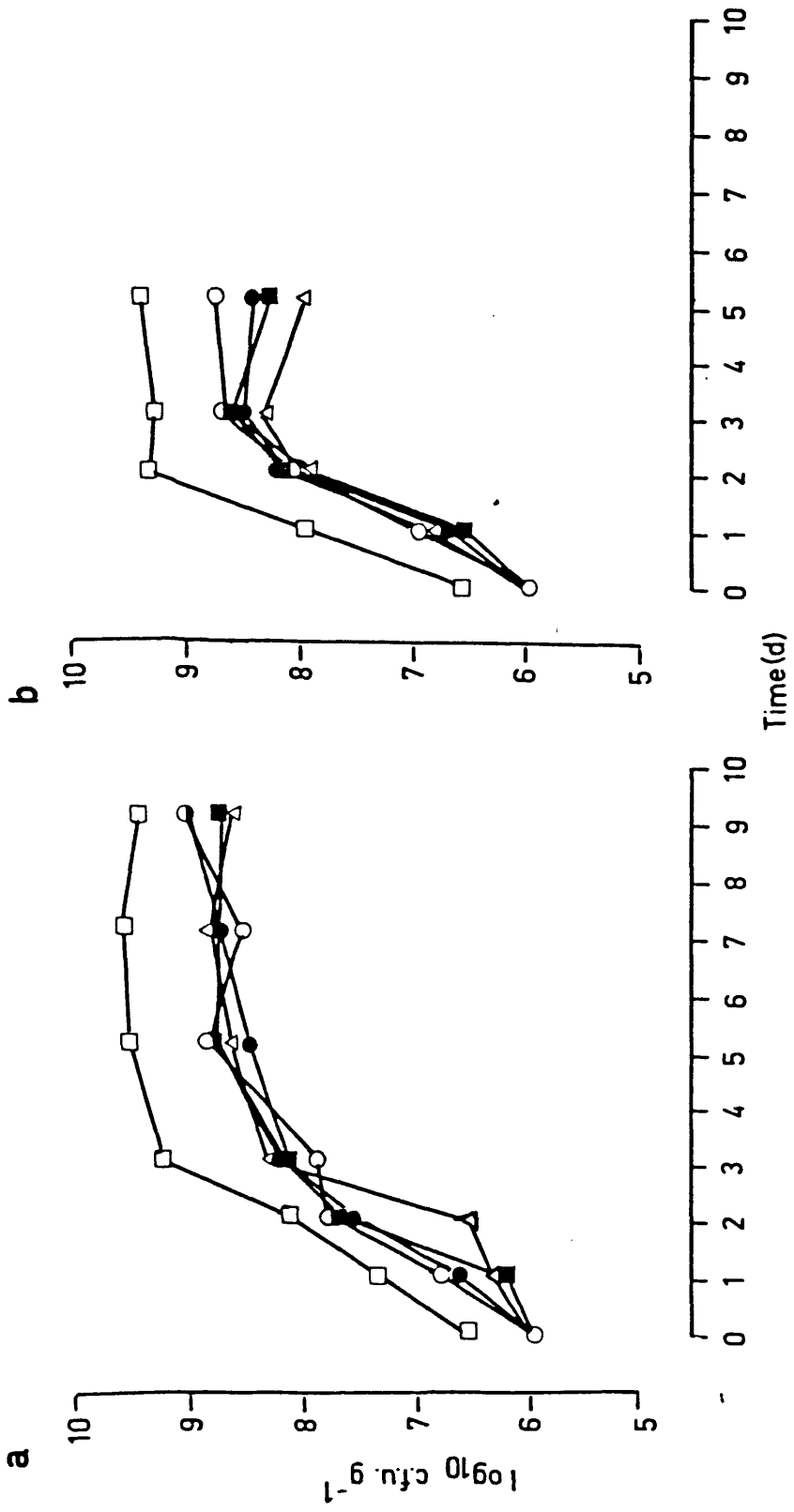
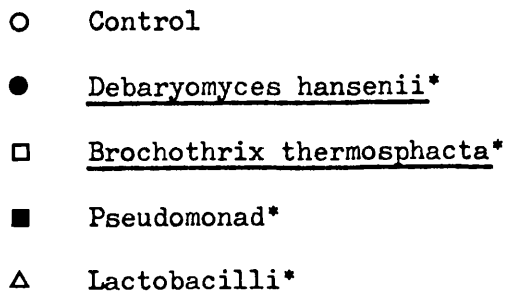

a
b
log10 c.f.u. g-1
10
9
8
7
6
5
0 1 2 3 4 5 6 7 8 9 10
Time(d)

Figure 35 The influence of inoculating sulphited (500 $\mu g\ g^{-1}$) pork belly mince with 4 microbial contaminants* on the rate of growth of lactobacilli during storage at (a) 1 and 15°C.



* Initial concentration ≥ 10^5 organisms g^{-1}

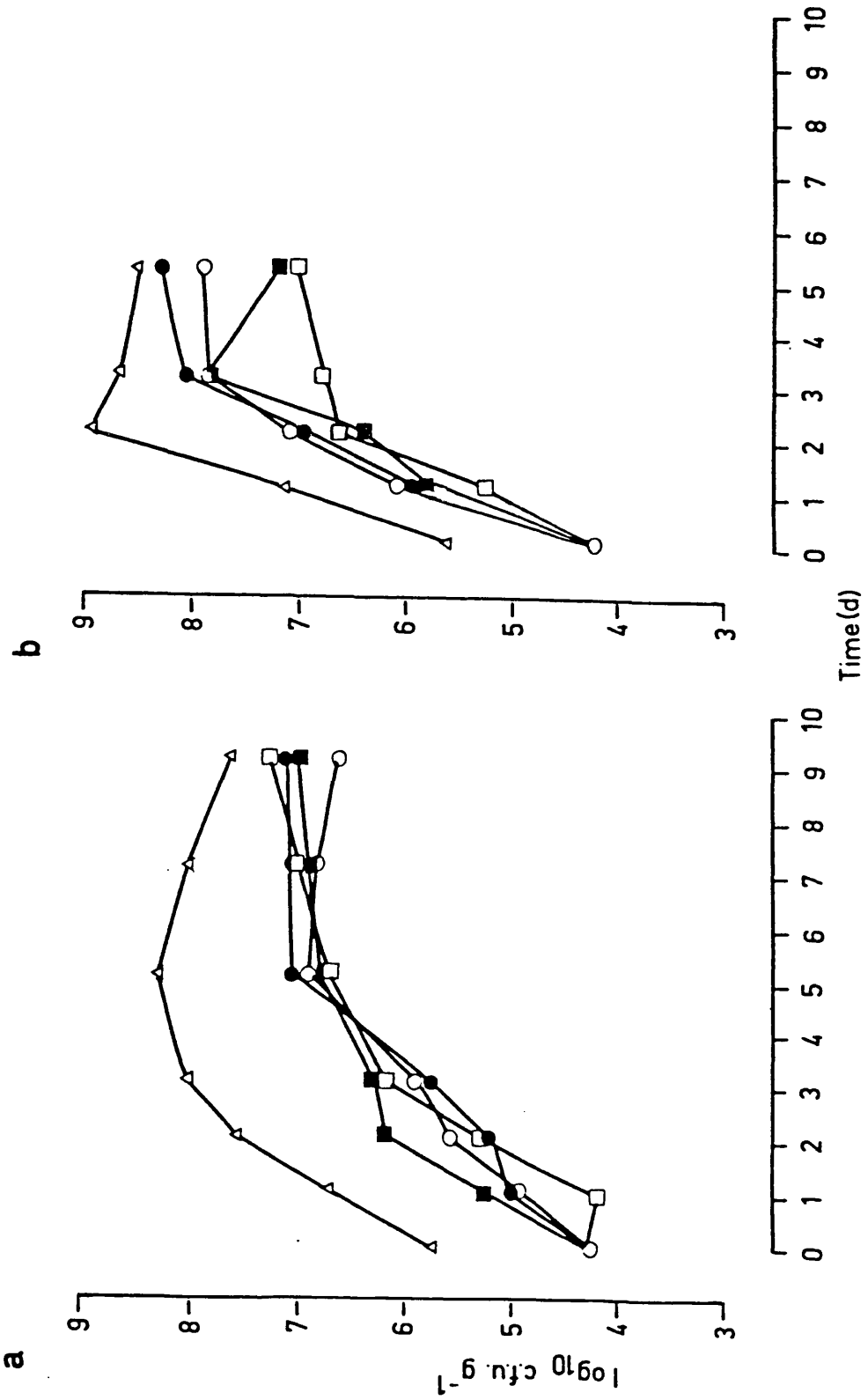
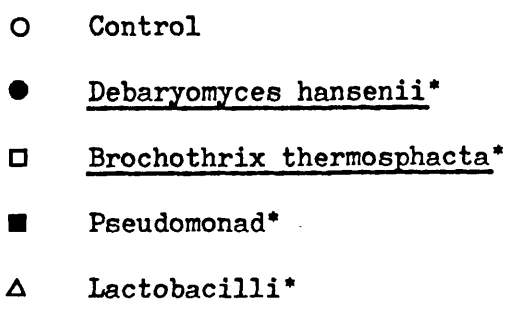

a
b
log10 c.f.u. g-1
Time(d)
0 1 2 3 4 5 6 7 8 9 10
3 4 5 6 7 8 9

Figure 36 The influence of inoculating sulphited (500 μg g^{-1}) pork belly mince with 4 microbial contaminants* on the rate of growth of pseudomonads during storage at (a) 1 and (b) 15°C.



* Initial concentration ▷ 10^5 organisms g^{-1}

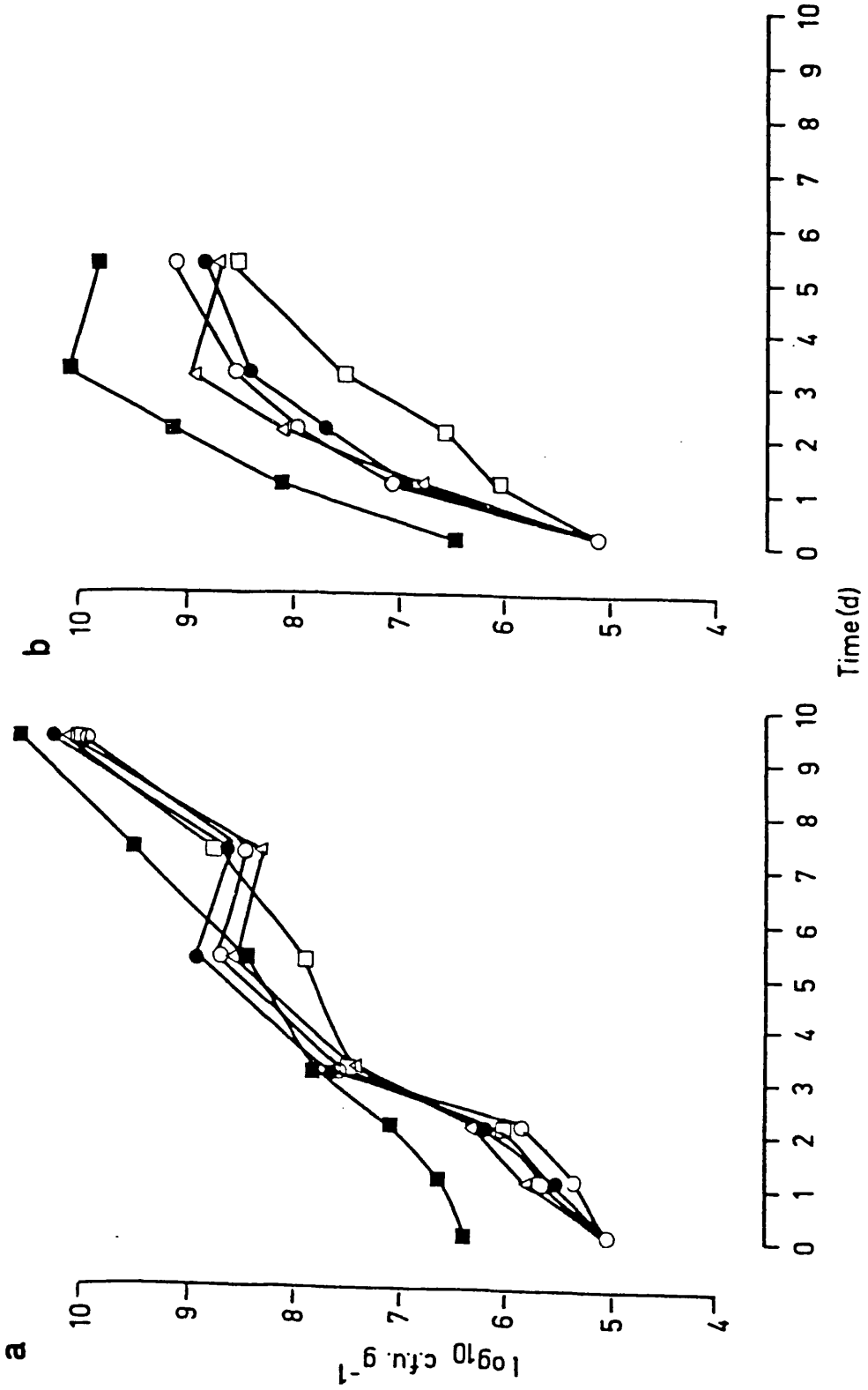
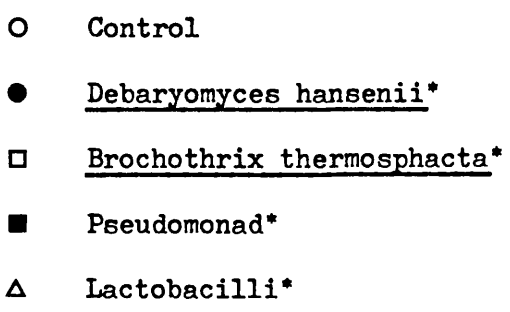

a
b
log10 c.f.u. g-1
Time(d)
0 1 2 3 4 5 6 7 8 9 10
4 5 6 7 8 9 10

Figure 37 The influence of inoculating sulphited (500 μg g^{-1}) pork belly mince with 4 microbial contaminants* on the rate of growth of enterobacteria during storage at (a) 1°C and (b) 15°C.



* Initial concentration ≥ 10^5 organisms g^{-1}

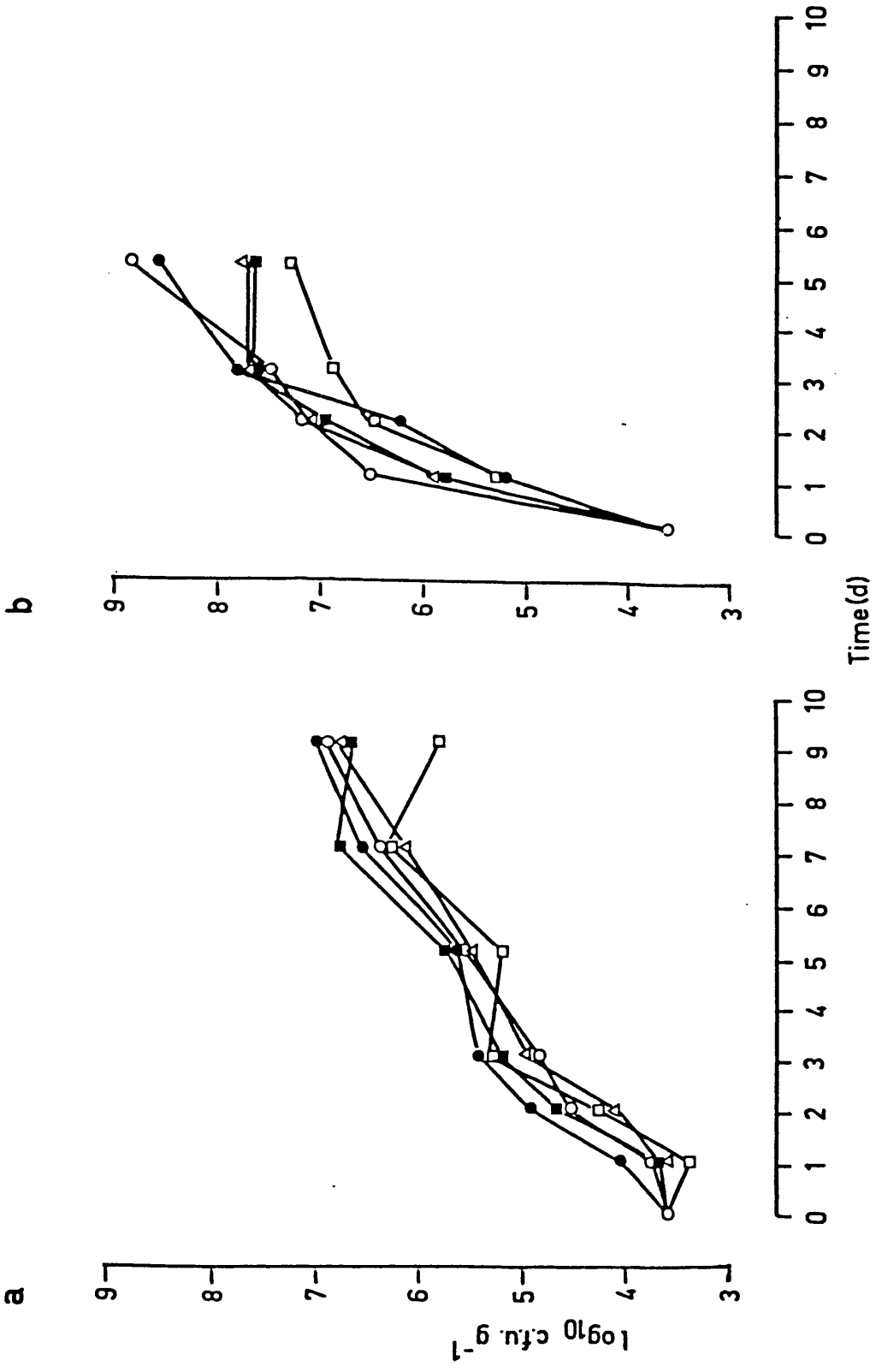

a
b
log10 c.f.u. g-1
9
8
7
6
5
4
3
0 1 2 3 4 5 6 7 8 9 10
Time(d)

Table 28 The influence of seeding sulphited pork belly mince with the yeast, Debaryomyces hansenii, Brochothrix thermosphacta, lactobacilli and pseudomonads on the climax populations (CP)and mean doubling time (MDT) of the microbrobial flora,

Sample	Yeast				Br.thermosphacta				Lactobacilli				Pseudomonads				Enterobacteria			
	CP		MDT		CP		MPT		CP		MDT		CP		MDT		CP		MDT	
	*1	15	1	15	1	15	1	15	1	15	1	15	1	15	1	15	1	15	1	15
Control	6.9	6.8	24.0	9.6	8.9	8.6	23.0	8.6	6.8	7.8	13.8	6.1	10.0	8.8	13.5	7.5	6.7	8.8	20.9	6.8
D.hansenii†	7.5	8.3	26.0	8.8	8.9	8.3	23.0	9.0	7.2	7.8	6.1	4.0	10.2	8.7	12.7	7.6	6.9	8.5	19.7	7.2
Br.thermo-sphacta†	6.8	6.75	6.3	9.6	9.5	9.25	24.0	8.2	6.8	6.8	15.0	8.3	10.1	8.4	12.8	10.7	6.3	7.2	24.5	10.6
Lactobacilli†	7.0	7.5	21.0	7.74	8.4	8.2	26.0	9.8	8.2	8.75	14.4	4.4	10.1	8.8	12.7	6.0	6.7	7.6	21.4	7.3
Pseudomonads†	6.8	6.8	24.0	9.0	8.75	8.5	24.0	8.6	6.9	7.2	13.0	6.4	10.5	10.0	17.5	6.2	6.7	7.6	21.0	7.3

* Storage temperature ^{o}C

† Mince belly inoculated with $> 10^{5}$ test organisms g^{-1}

Figure 38 The influence of inoculum concentration of the acetaldehyde producing yeast, Debaryomyces hansenii, on the growth of yeast in sulphited (500 $\mu g\ g^{-1}$) minced pork belly during storage at (a) 1 and (b) 15°C.

○ Control

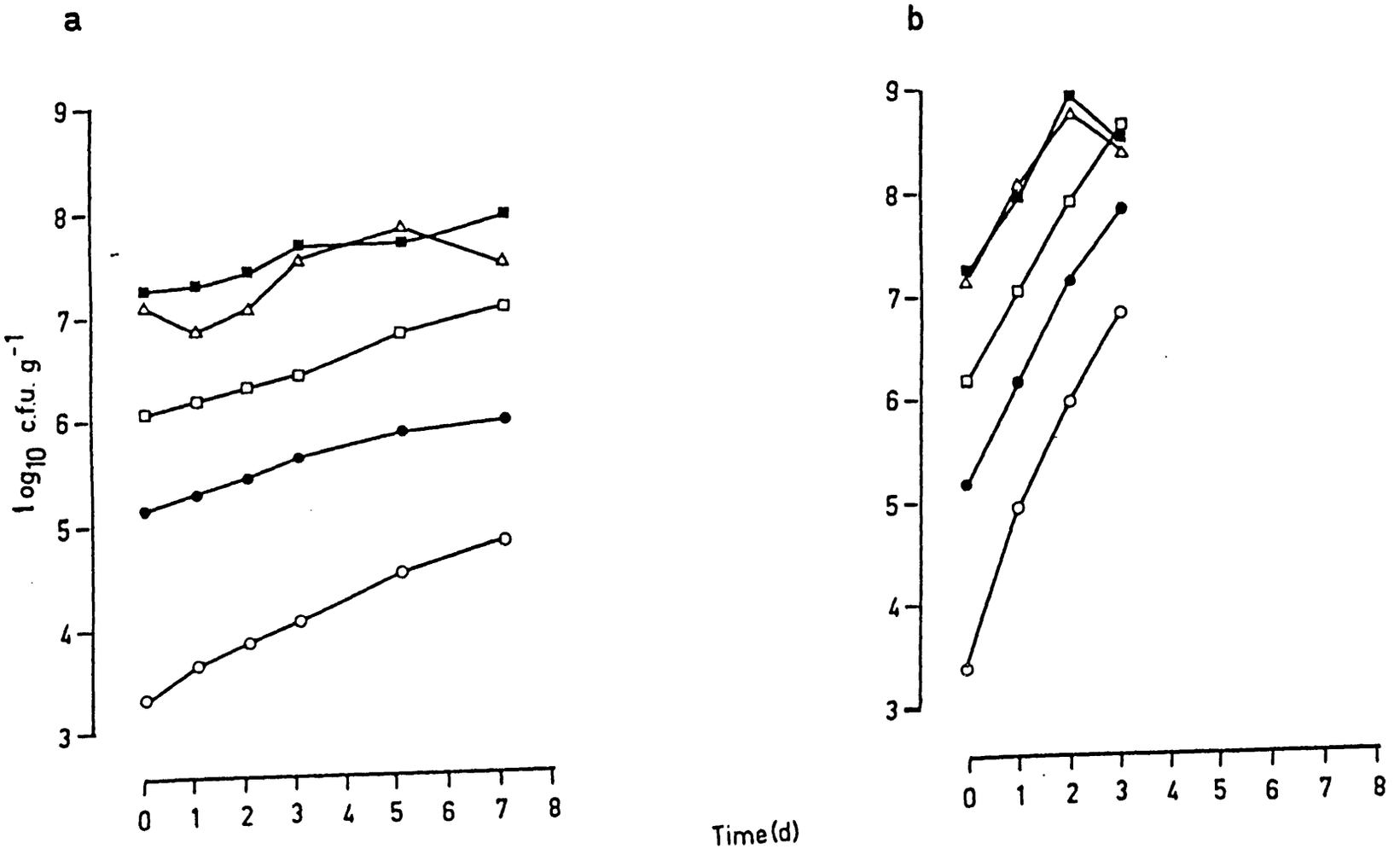
● *$10^5\ g^{-1}$

□ $10^6\ g^{-1}$

■ $10^7\ g^{-1}$

△ $10^7\ g^{-1}$

* Initial concentration of the yeast inoculum


a
b
log10 c.f.u. g-1
9
8
7
6
5
4
3
0 1 2 3 4 5 6 7 8
Time (d)

pH

The pH of none of the samples varied appreciably from the control during storage at 1°C (Figure 39, 40). During storage at 15°C there was a more pronounced acid drift in samples seeded with Br. thermosphacta, a lactobacillus or a pseudomonad (ca 1.1 - 1.4 units) than in the control or samples seeded with yeast (ca 0.5 - 0.17 units). Indeed, the size of the yeast population did not influence appreciably the pH of the pork belly mince stored at either temperature (Figure 40).

Fate of sulphite

With storage at 1 and 15°C the concentrations of free and total sulphite diminished rapidly in all of the seeded mince samples (Figure 41 - 44). Thus, the trends were similar to those observed previously (pp 147-151). Likewise, after an initial increase, the concentrations of bound sulphite decreased markedly during storage. The extent of sulphite binding in samples seeded with D. hansenii was appreciably higher (30 $\mu g\ g^{-1}$ and 20 $\mu g\ g^{-1}$ at 1 and 15°C respectively) than in the controls and also minced meat inoculated with the other organisms (Figures 41, 42). Indeed, the concentrations of bound sulphite attained in samples seeded with other organisms were either lower, e.g. Br. thermosphacta and pseudomonad seeded samples stored at 15°C and also lactobacilli seeded samples stored at 1°C, or not appreciably different from the control. Moreover, the rate and

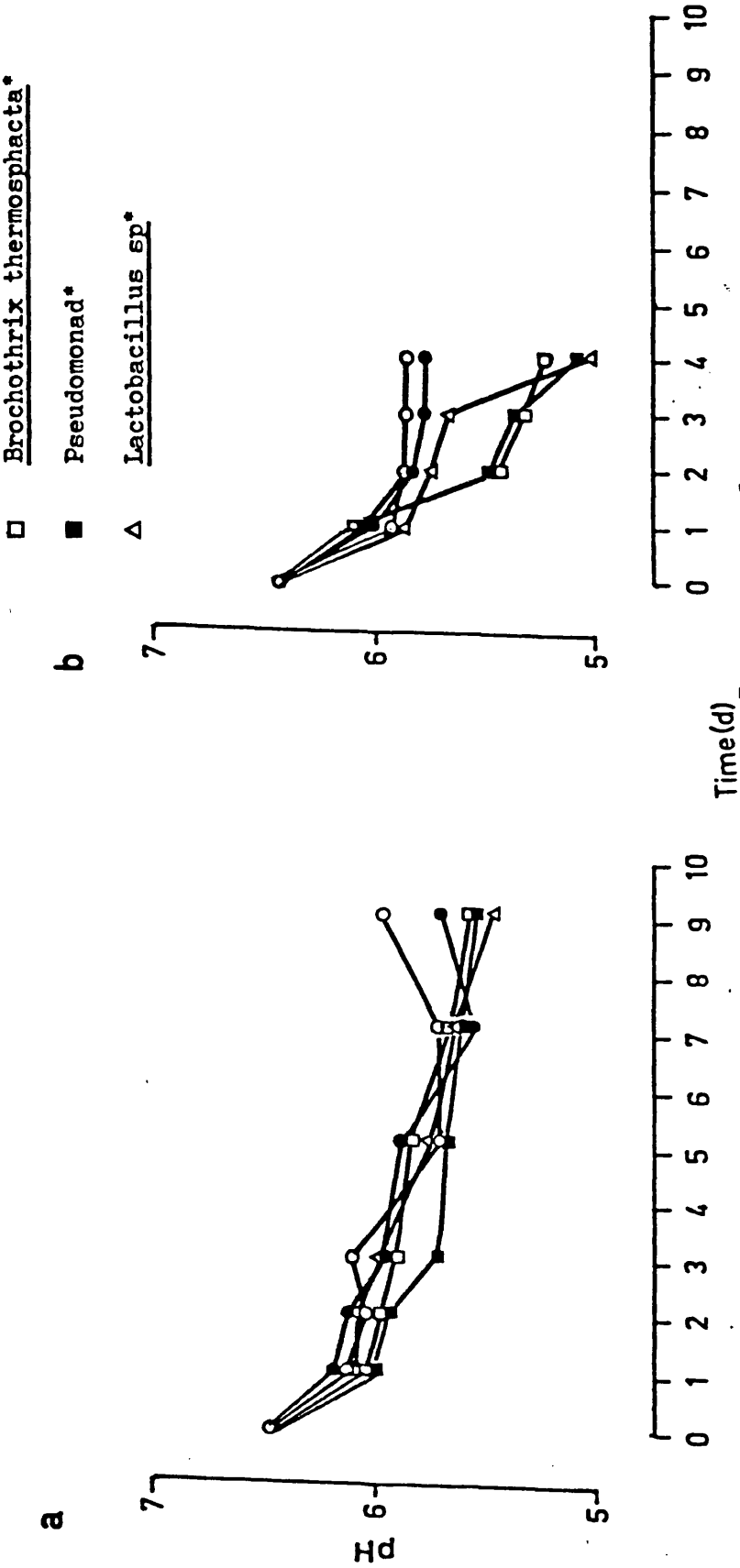


Figure 39 The influence of inoculating sulphited (500 µg g^{-1}) minced pork belly with 4 microbial contaminants (initial concentration > 10^5 g^{-1}) on the pH of homogenates during storage at (a) 1 and (b) 15°C.

Figure 40 The influence of the inoculum concentration of the acetaldehyde producing yeast Debaryomyces hansenii on the pH of sulphited (500 µg g^{-1}) minced pork belly homogenates during storage at (a) 1 and (b) 15°C.

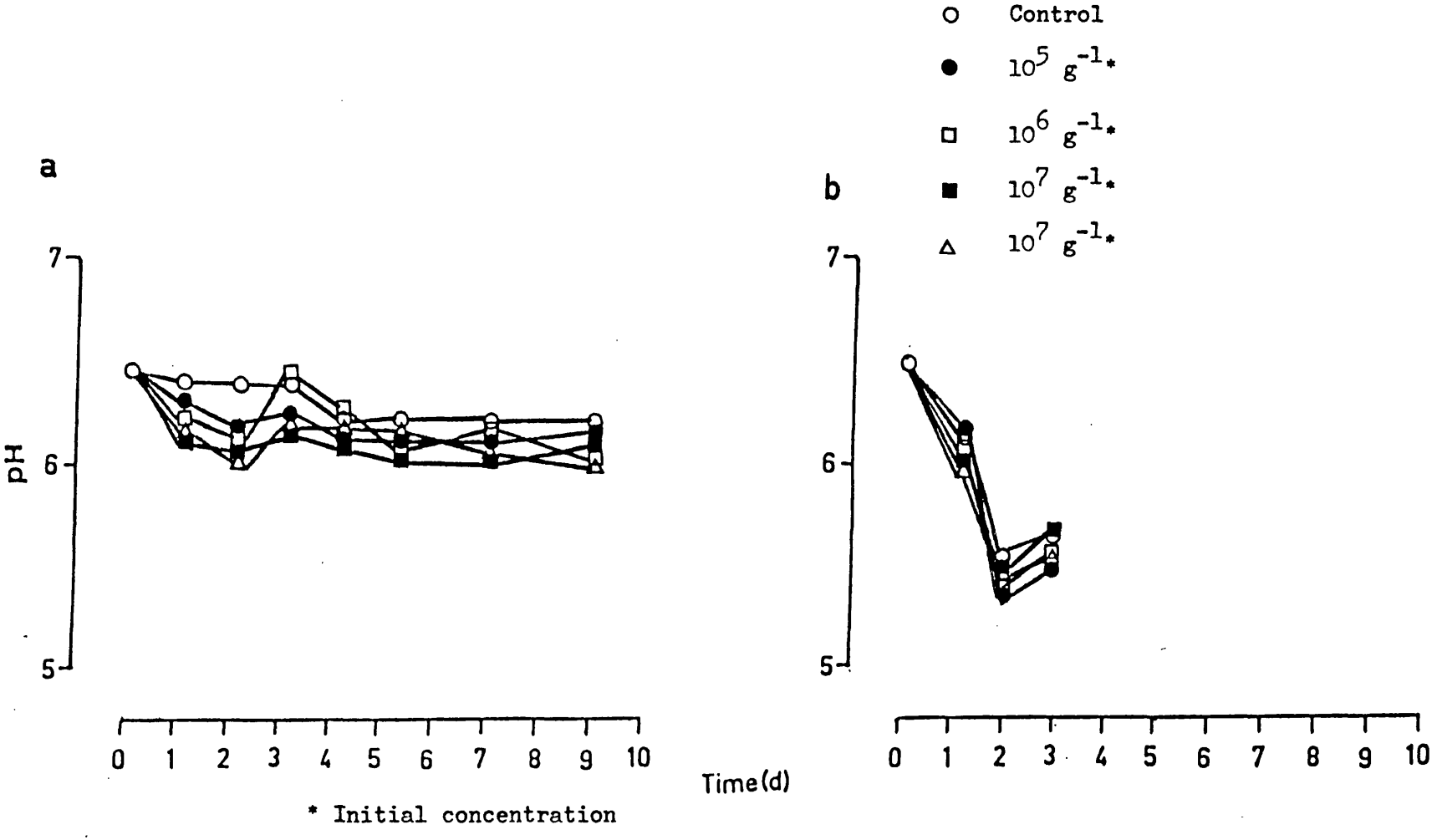


Figure 41 The influence of inoculating sulphited (500 $\mu g\ g^{-1}$) minced pork belly with 4 micro-organisms on the fate of (a) total, (b) free and (c) bound sulphite during storage at $1^{\circ}C$.

- ← Amount added initially
- ○ Control
- ● Debaryomyces hansenii*
- □ Brochothrix thermosphacta*
- ■ Pseudomonads*
- △ Lactobacillus sp*

* Initial concentration $> 10^5$ organisms g^{-1}

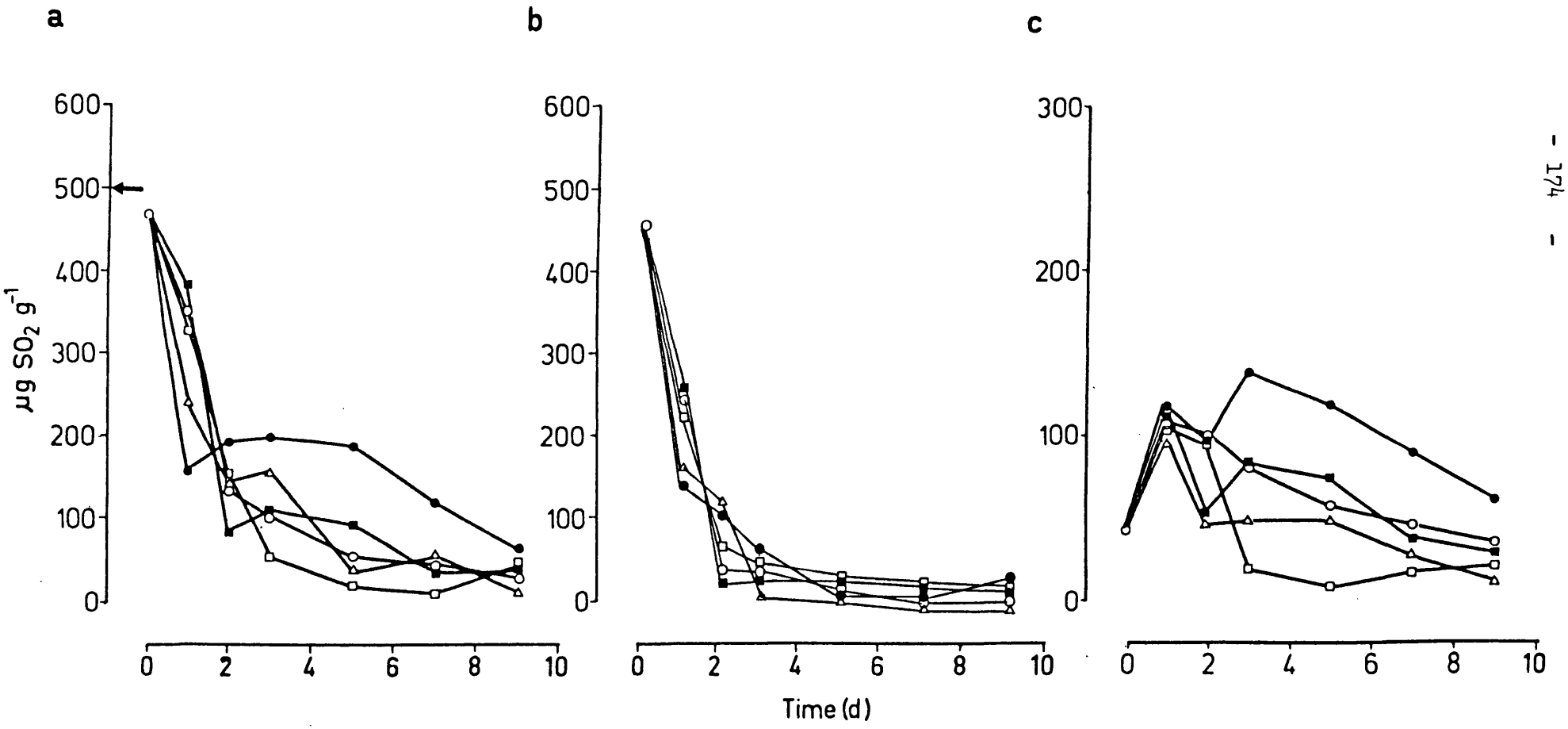

a
b
c
600
500
400
300
200
100
0
300
200
100
0
µg SO2 g-1
0
2
4
6
8
10
Time (d)

Figure 42 The influence of inoculating sulphited (500 $\mu g\ g^{-1}$) minced pork belly with 4 micro-organisms on the fate of (a) total, (b) free and (c) bound sulphite during storage at $15^{o}C$.

← Amount initially added

○ Control

● *Debaryomyces hansenii**

□ *Brochothrix thermosphacta**

■ Pseudomonads*

△ *Lactobacillus* sp*

* Initial concentration > 10^{5} organisms g^{-1}

a
b
c
µg SO2 g-1
Time (d)
0
100
200
300
400
500
600
2
4
6

Figure 43 The influence of the concentration of the acetaldehyde producing yeast, Debaryomyces hansenii, on the fate of (a) total, (b) free and (c) bound sulphite in minced pork belly during storage at 1°C.

← Amount added initially

○ Control

● 10^5 g^{-1}*

□ 10^6 g^{-1}*

■ 10^7 g^{-1}*

△ 10^7 g^{-1}*

* Initial concentration of yeast

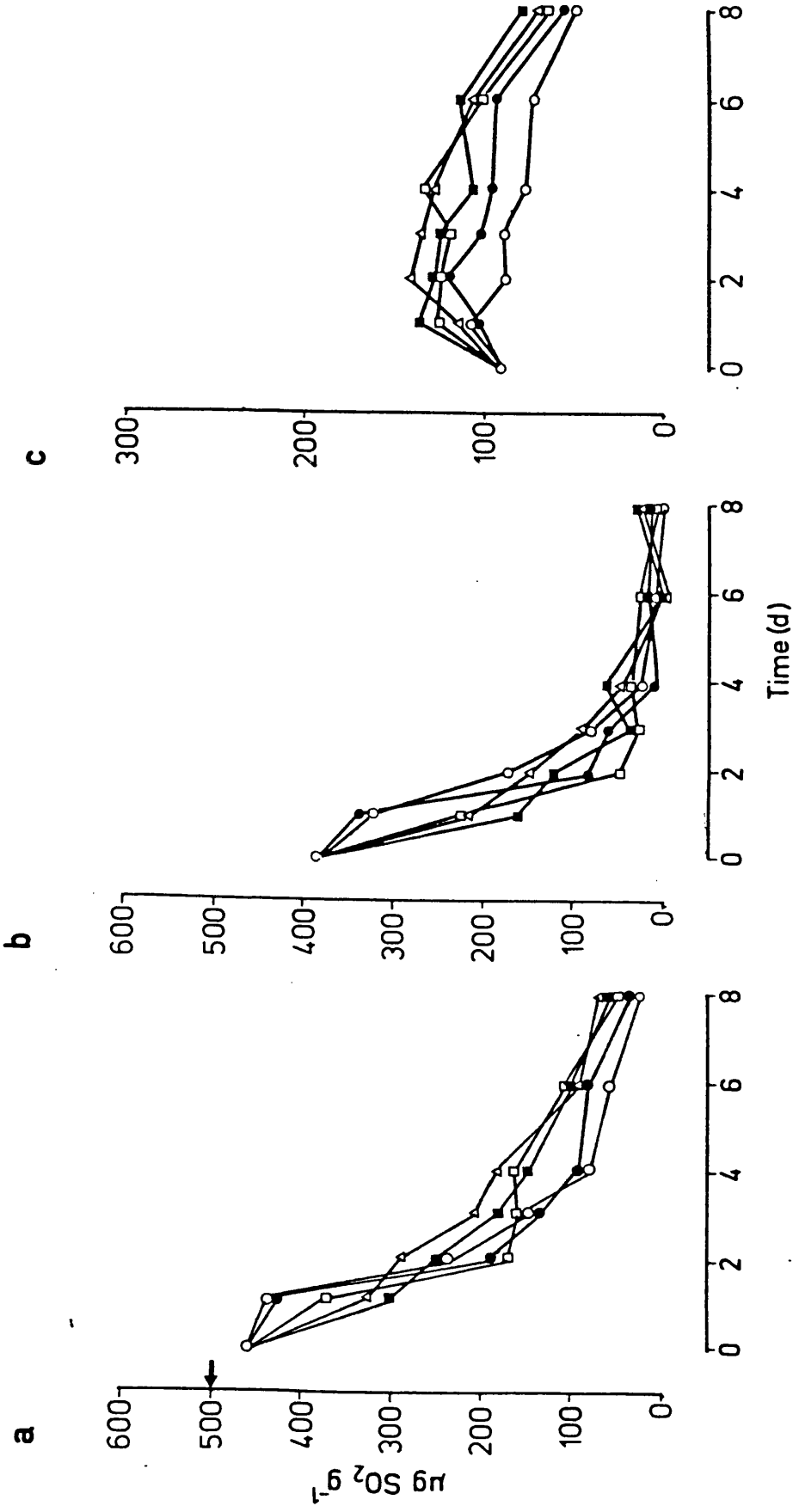
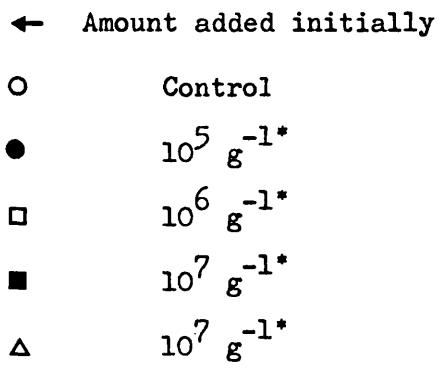

a
b
c
µg SO2 g-1
600
500
400
300
200
100
0
0
2
4
6
8
Time (d)

Figure 44 The influence of the concentration of the acetaldehyde producing yeast, Debaryomyces hansenii, on the fate of (a) total, (b) free and (c) bound sulphite in minced pork belly during storage at 15°C.



* Initial concentration of yeast

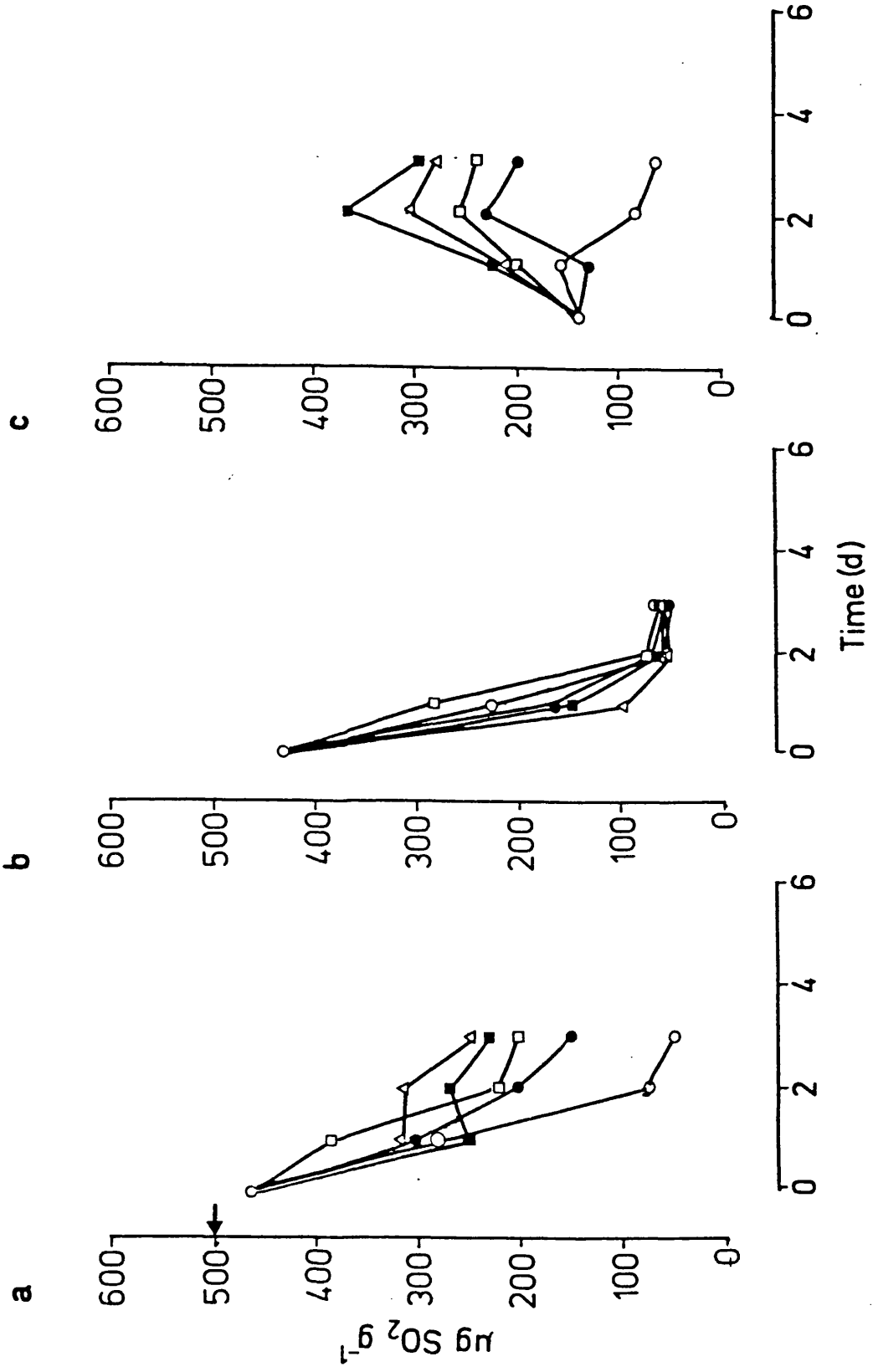
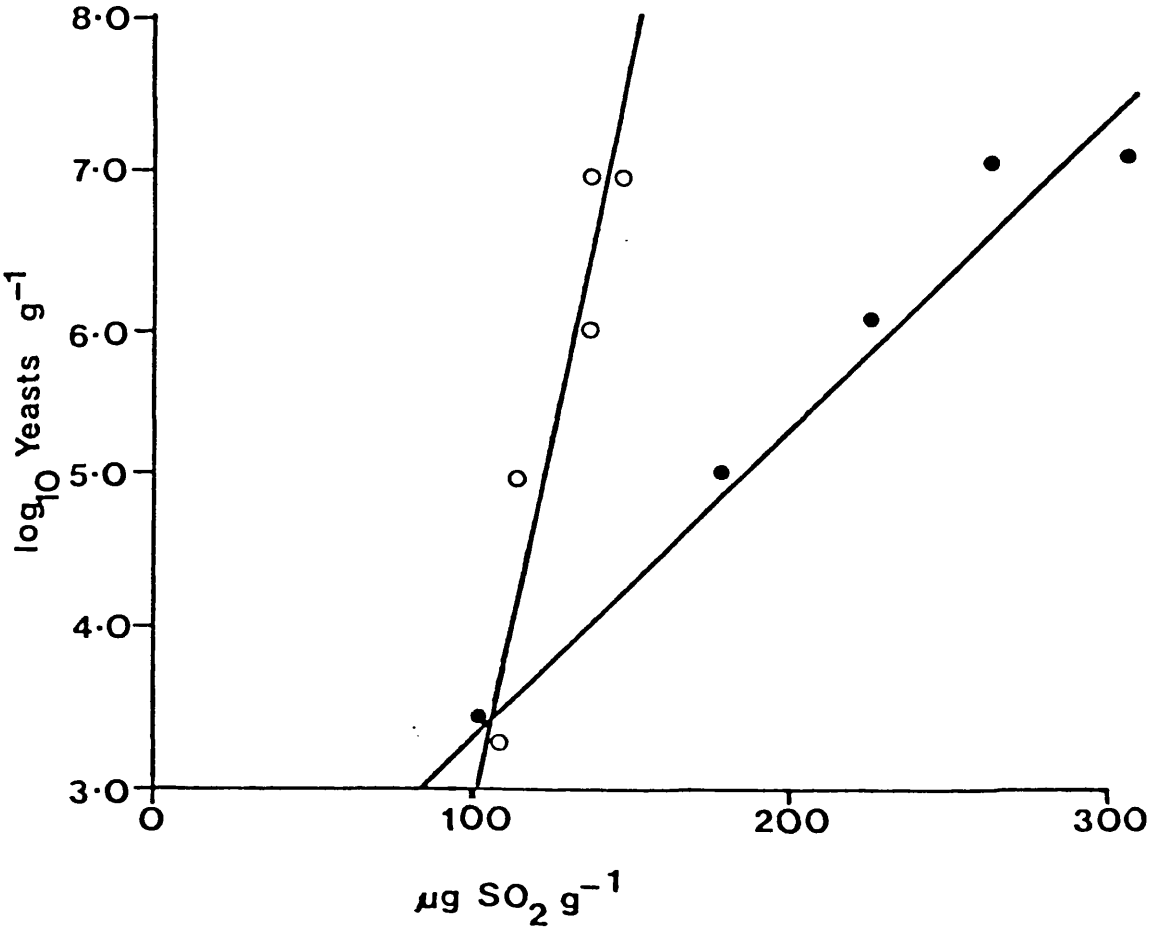

a
b
c
µg SO2 g-1
600
500
400
300
200
100
0
0
2
4
6
Time (d)

Figure 45 Regression analysis of the initial concentration of the acetaldehyde producing yeast, Debaryomyces hansenii, and the concentration of bound sulphite in minced pork belly during storage at 1 °C (O, r = 0.98) and 15°C (●, r = 0.92)



extent of binding during storage at either temperature in samples seeded with D. hansenii increased concomitantly with an increase in the size of the initial inoculum (Figures 43, 44). Indeed, the concentration of bound sulphite in samples inoculated with $>10^7$ yeasts g^{-1} were 20 $\mu g\ g^{-1}$ and 200 $\mu g\ g^{-1}$ higher than the control sample following storage at 1 and 15°C respectively. Regression analysis revealed that these values were positively related ($r = 0.98$ at 1°C and $r = 0.92$ at 15°C Figure 45). In every instance the rate and extent of binding was greatest in samples stored at 15°C. These findings suggest that of the major microbial contaminants of mince pork only yeast possess the ability to enhance the extent of sulphite binding.

(3) Sulphite binding in sausage

This part of the study was undertaken with the objective of determining the concentrations of acetaldehyde and other binding agents in sausages obtained directly on two occasions from a local manufacturer or from retail outlets. In agreement with previous findings, both in this study (pp 82, 83) and also those of Banks (1983), the concentrations of total sulphite remained relatively constant throughout storage (Figure 46), whereas those of free sulphite diminished and those of bound sulphite increased, particularly at the highest (15°C) storage temperature. Of the potential binding agents

Figure 46

(a) The concentration of free (○)[1], bound (●)[1] and total (□)[1] sulphite, acetaldehyde (■)[2], pyruvate (△)[3] and ∝ ketoglutarate (▲)[4] in a batch of sausages stored at 1 and 15°C. (Experiment 1)

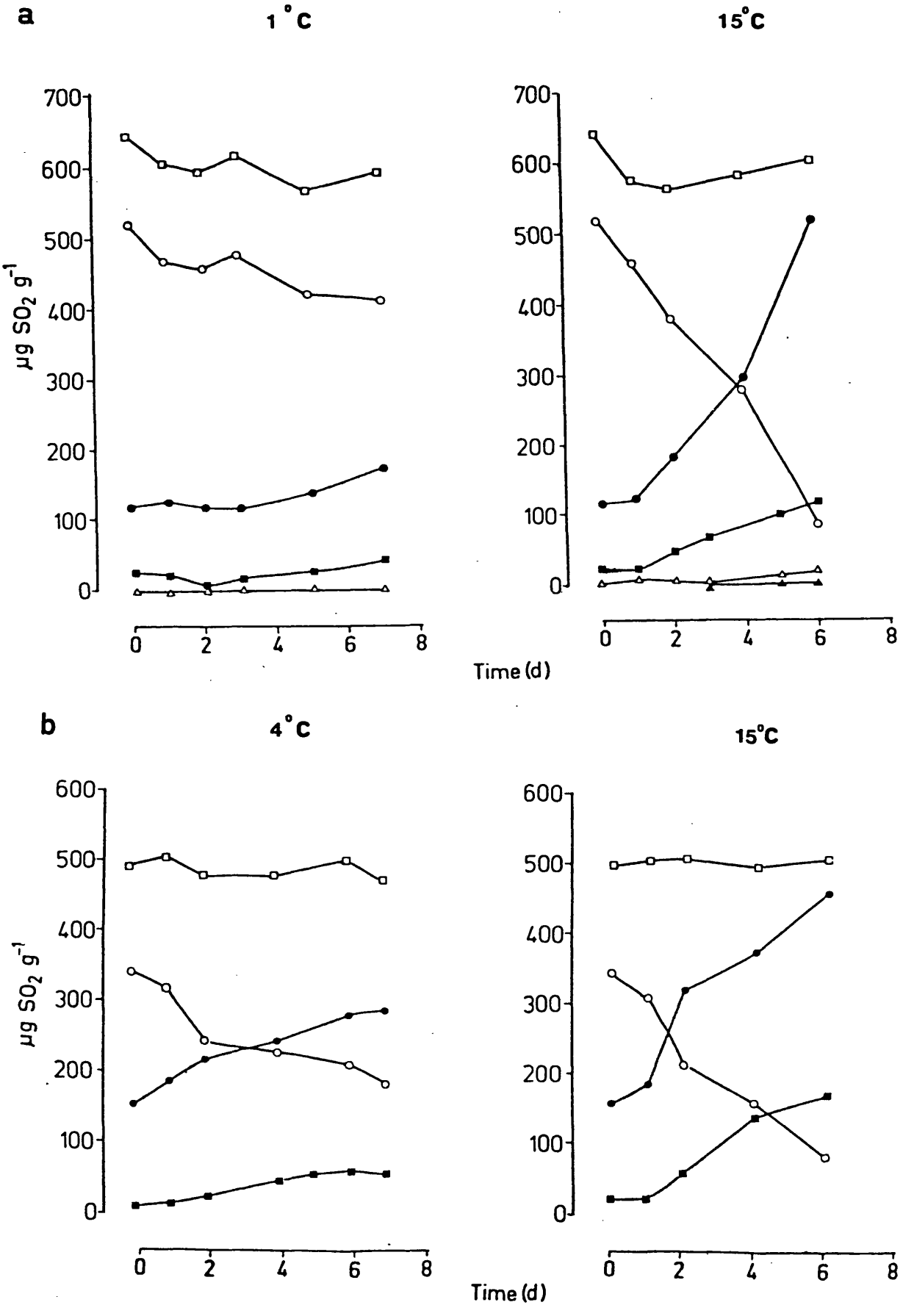
(b) The concentration of free (○)[1], bound (●)[1] and total (□)[1] sulphite and acetaldehyde (■)[2] in a batch of sausages stored at 4 and 15°C. (Experiment 2)

1 Determined by the method of Banks and Board (1982a).

2 Determined by the enzymic method of Bernt and Bergmeyer (1974a)

3 Determined by the Boehringer Mannheim test combination kit for puruvate.

4 Determined by the enzymic method of Bernt and Bergmeyer (1974b).


a
1 °C
15°C
700
600
500
400
300
200
100
0
µg SO2 g-1
0
2
4
6
8
Time (d)
b
4°C
15°C
600
500
400
300
200
100
0
µg SO2 g-1
0
2
4
6
8
Time (d)

produced by yeasts (Table 25) acetaldehyde, was again the major one in sausage, its concentrations increasing during storage at 1, 4 and 15°C (Figure 46). The concentrations of pyruvate and α ketoglutarate were negligible. The rate of increase of the concentration of acetaldehyde was greatest at the higher storage temperature. This probably reflects the rate and extent of yeast growth (Figure 47, experiment 1); both increased appreciably with storage at a higher temperature. In other words, as with yeasts growing in sulphited lab lemco broth (Figure 17 - 22), the rate of acetaldehyde synthesis during storage at 15°C (Experiment 1) was maximal during the log phase of yeast growth. The proportion of acetaldehyde-bound sulphite increased to 40 and 70% following storage at 1 and 15°C (Figure 48). Regression analysis of the concentration of acetaldehyde and the extent of sulphite binding in sausages which had been stored as well as those obtained from retail outlets (Figure 49) revealed that the values were positively related ($r = 0.89$, $p = <0.05$). These findings are taken to mean that acetaldehyde is an important but not the sole sulphite binding agent in sausage. Concentrations of acetaldehyde far in excess of that required to bind all the sulphite were recovered from sausages obtained from retail outlets containing exceptionally high concentrations of sulphite ($>$ 700-900 $\mu g\ g^{-1}$; Figure 49).

Figure 47 The growth of yeasts in sausages stored at 1 and 15°C (Experiment 1);

○ 1°C

● 15°C

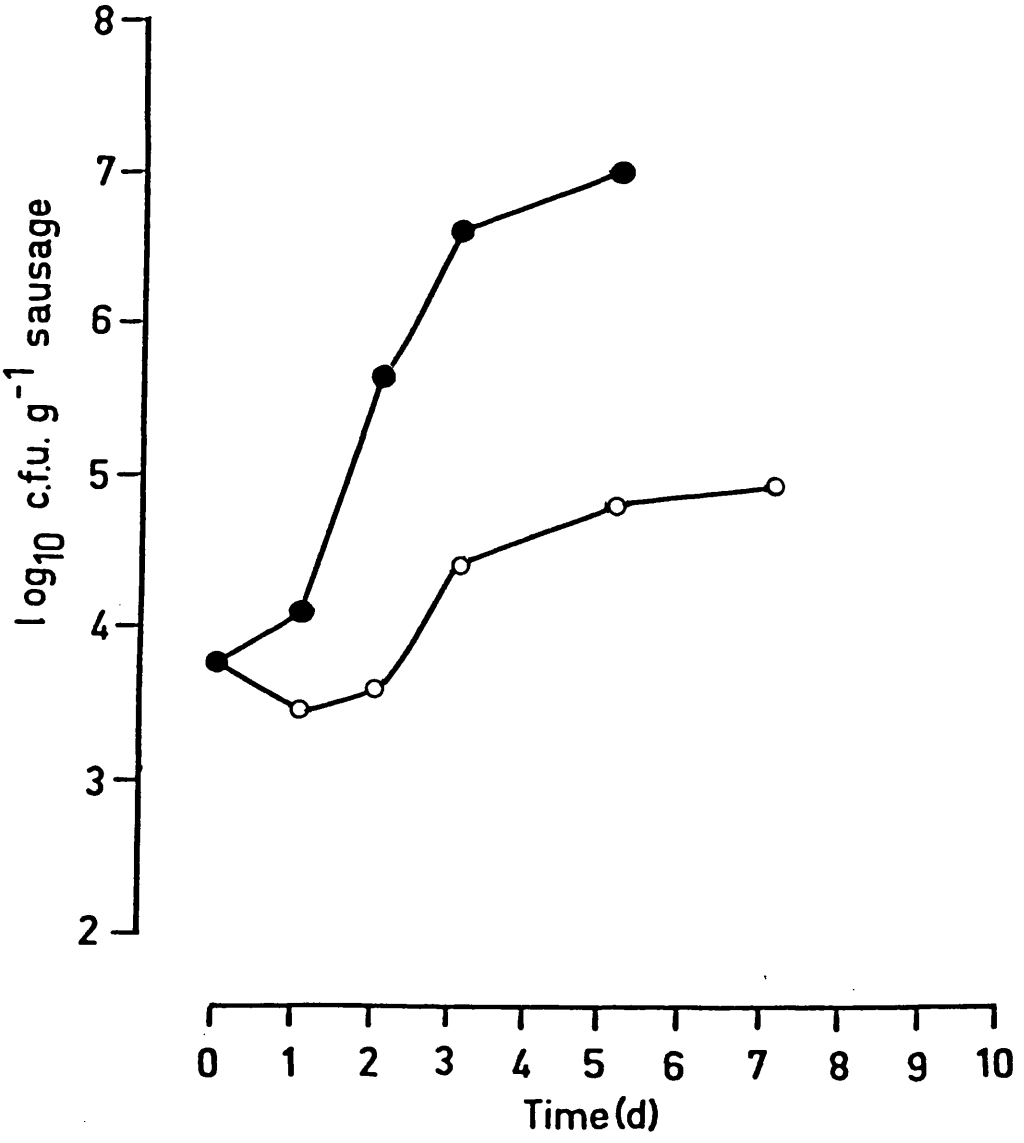
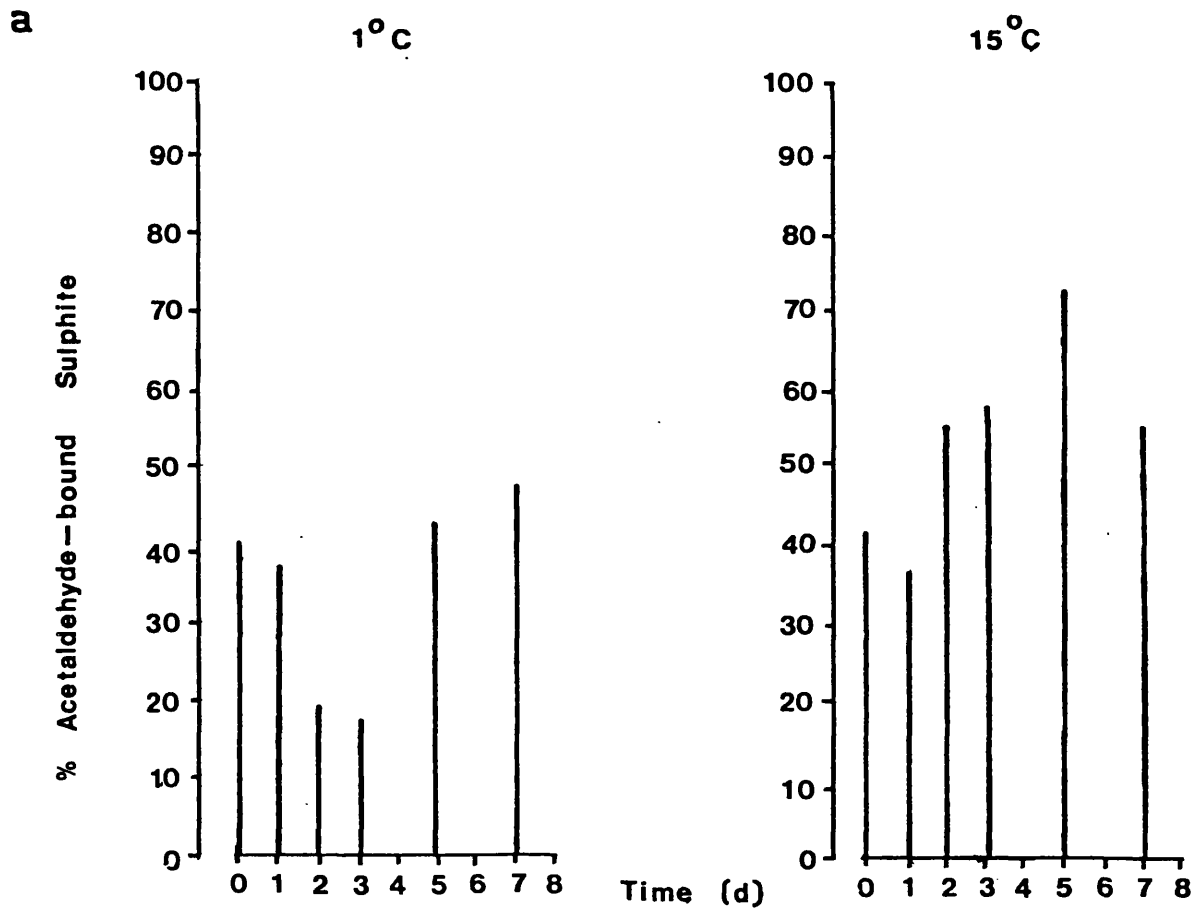


Figure 48 The percentage* of acetaldehyde-bound sulphite in pork sausage during storage :-

(a) Experiment 1 (p 184)

(b) Experiment 2 (p 182)

* Estimated by assuming the binding of acetaldehyde and sulphite to be 1 : 1 (Burroughs and Sparks, 1973).


a
1°C
15°C
% Acetaldehyde – bound Sulphite
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8
Time (d)

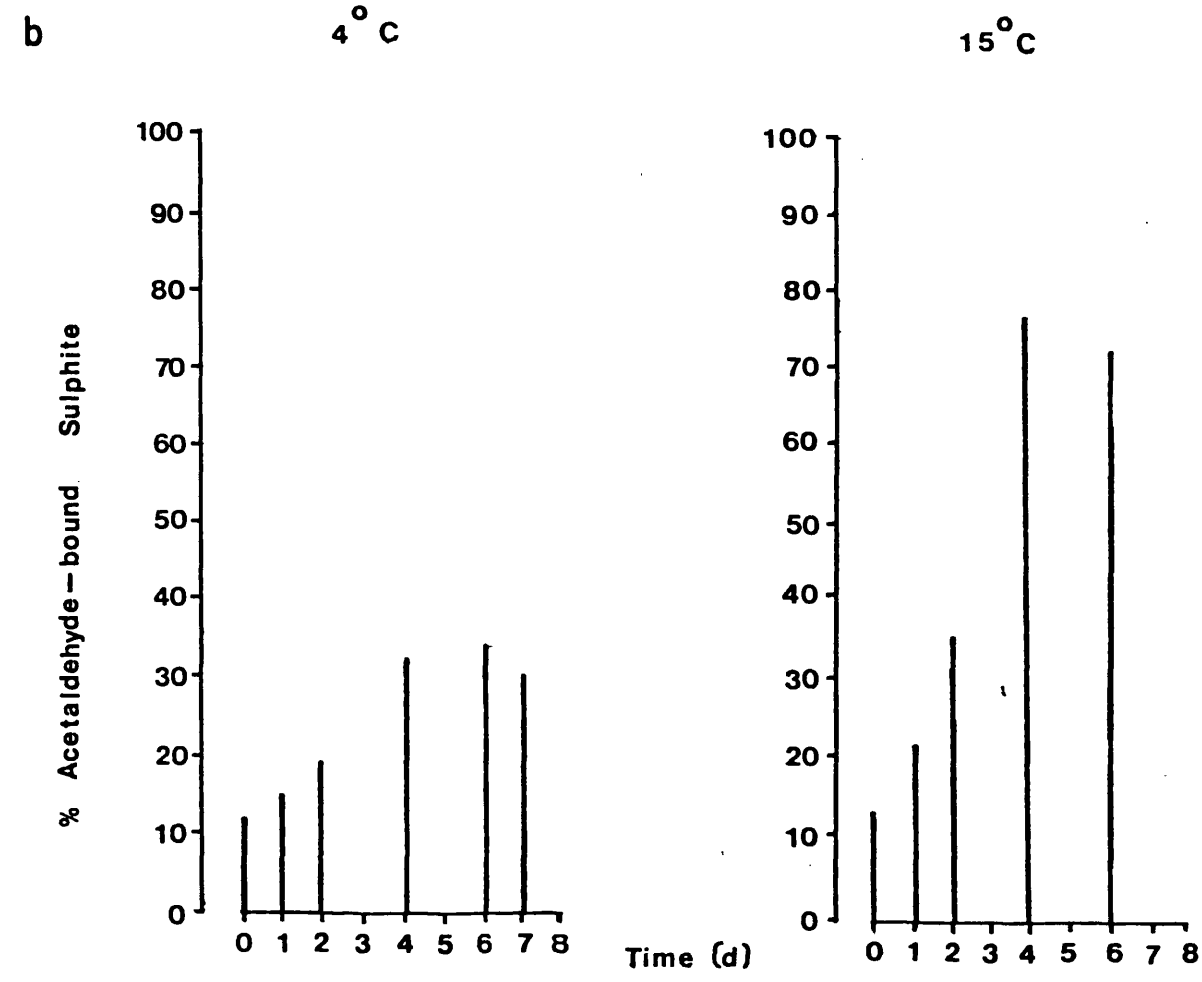
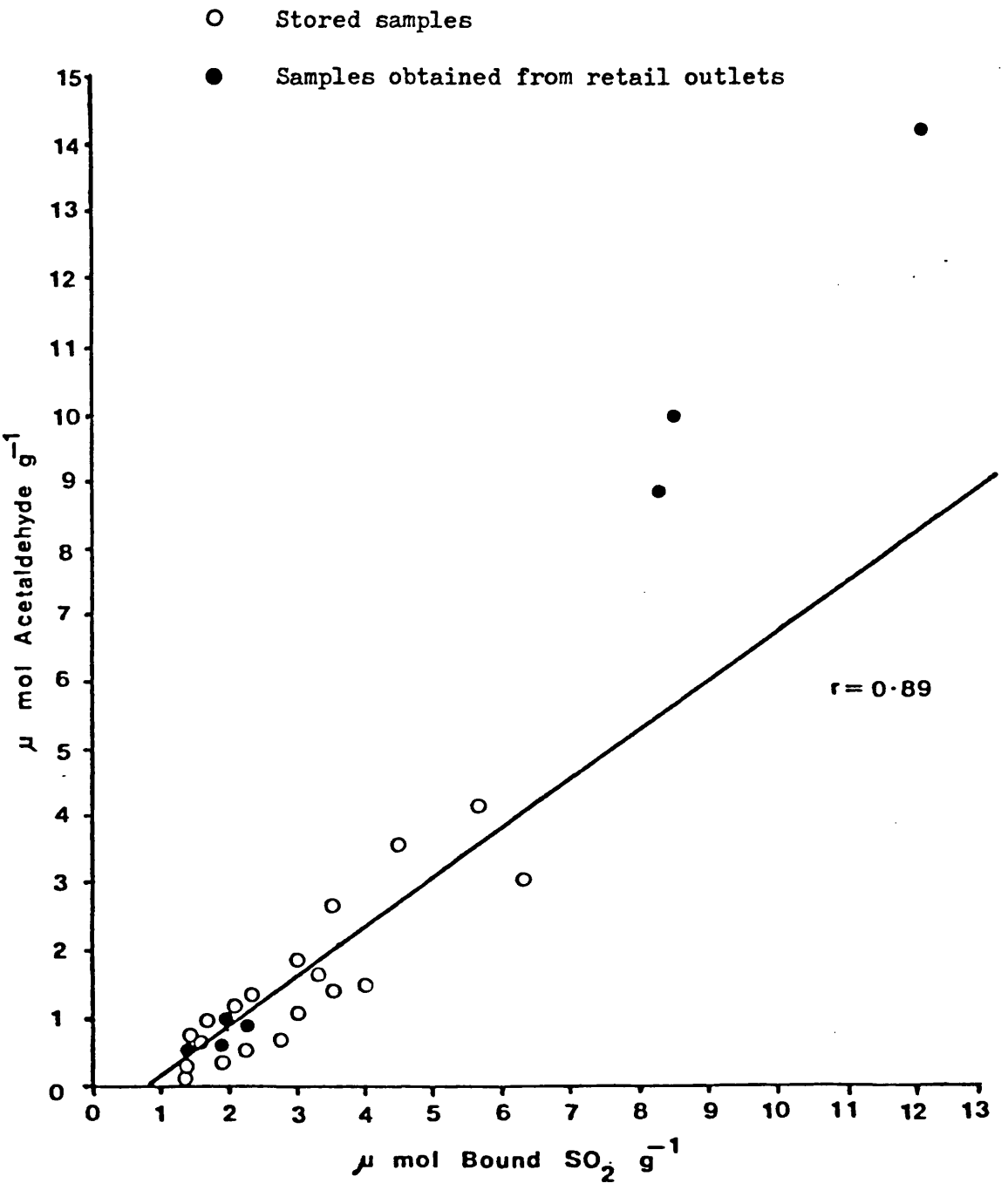

b
4° C
15°C
% Acetaldehyde – bound Sulphite
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8
Time (d)

Figure 49 Regression analysis of the concentrations of acetaldehyde[1] and bound sulphite[2] in pork sausages.



1 Determined by the enzymic method of Bernt and Bergmeyer (1974a).

2 Determined by the method of Banks and Board (1982a).

DISCUSSION

DISCUSSION

Earlier studies have indicated that, in terms of numbers, yeasts must be regarded as one of the contributors to the microbial association of the British fresh sausage (Dowdell and Board, 1971). The findings of this survey are in accord with these studies. Indeed, even though they were not the numerically dominant organism, estimates of the relative mass of yeasts (assumed to be a prolate spheroid) and the principal bacterial contaminant, Br. thermosphacta (assumed to be a cylinder), revealed that the former's contribution to the biomass of sausage was 3 times that of the latter. Moreover, comparisons of regression analysis (pp 76 - 80) of the numbers of yeasts and general contaminants; principally Br. thermosphacta in sausage revealed that the rate of increase of the sizes of the populations of both were of the same order. Such a situation was not obtained in unsulphited minced meat. Thus, it can be assumed that the yeasts are major components of the microbial association of sausages and that their growth in sausages is favoured by sulphite. Indeed, it may be presumed that they probably contribute to the changes in the product during storage and may, thus, be associated with spoilage. This situation contrasts with that of minced and chilled joints of meat, the principal source of yeast contamination in sausage (Table 20). It is generally accepted that yeasts grow more slowly than bacteria and, as a consequence, are quickly outgrown by psychrotrophic bacteria in refrigerated meats.

The review of Walker and Ayres (1970) concluded that yeasts were only considered to play a major role in spoilage when the bacterial populations had been arrested in some way, as has been demonstrated in chlortetracycline-treated poultry (Njoko-Obi, et al., 1957; Wells and Stadelman, 1958; Walker and Ayres, 1959). The findings of this survey and also those of Banks (1983) have shown that the growth of yeasts in sausages is unimpaired by legally permitted levels of sulphite. Indeed, of the members of the association, the yeasts appear to have the greatest tolerance of the preservative. It is probable that sulphite, which is known to impede the growth of the nutritionally non-fastidious bacteria, pseudomonads, for example, that dominate the flora of carcass and minced meat (Banks and Board, 1981) may contribute to the establishment of the yeast flora by reducing competition.

As was noted in the Introduction, an understanding of the microbiological and related chemical changes in sausages is being hindered by a lack of information about the taxonomy and source of yeast contaminants and also about the physiological attributes of these organisms, especially in respect of their suspected interaction with sulphite. The results section dealt with several of these features and my observations have identified 3 topics for discussion : (1) the taxonomic problems associated with the study of yeasts obtained from meat and meat products; (2) the physiological attributes that could be expected

to contribute to spoilage of sausages and (3) the biochemistry of sulphite binding.

(1) Taxonomy

Perusal of the literature on the microbiological contamination of meat and meat products permits the conclusion that little attention has been given to the taxonomy of the yeasts. There are probably two reasons for this. Firstly, the available literature gives the impression that the majority of meat microbiologists consider the identification of the yeasts isolated from meat and meat products is unnecessary because they are rarely, if ever, associated with spoilage. Secondly, yeast taxonomy, which is principally traditional in format (Lodder, 1970), is considered, by many, to be confusing, complex as well as time consuming and, therefore, to be avoided. In practice, of course, the term yeast is applied to a very heterogeneous group of organisms, having one unifying property, the unicellular state (Lodder, 1970). Indeed, the complexity of yeast taxonomy is a direct consequence of the fact that a wide range of unicellular fungi have the characteristics associated with one or other of the three major groups, ascomycetous, basidiomycetous or fungi imperfecti (Lodder, 1970). Consequently, there are few common properties (Davenport, 1975) upon which to base the definition of species or a general classification. The taxonomy of yeasts contrasts with bacteria

also. Rarely, if ever, are the latter considered as a whole for taxonomic purposes. As was done in this study, selective and, in some instances, differential media are used for the isolation of major groups of bacteria and the characterisation is done by selected methods that long experience has shown to be appropriate for particular groups. Indeed, this is the basis of the genera defined in Bergey's(1974) manual. The diversity of the yeasts makes such a system impossible and, in the absence of selective and differential media, characterisation normally proceeds *via* preliminary delimitation into broad groups on the basis of morphological and physiological parameters. Thereafter, more specific physiological and biochemical attributes can be analysed and the identity of a species established (Lodder, 1970). Such a system is time consuming and precise definitions are often confused in the case of yeasts having life cycles (Davenport, 1980). These problems and also the period of time required to obtain results, as a consequence of their slower growth rate *vis-à-vis* bacteria, has made yeast identification, particularly for a large number of isolates, an unattractive proposition.

In the course of this study a total of 717 isolates from sausage, ingredients, minced beef and a local factory were identified according to the criteria and definitions proposed by Lodder (1970). The extremely high reproducibility (*98.75%*) of the results obtained with 66 isolates sampled on two occasions and also stock cultures from NCYC would suggest that the

identification scheme used was sufficiently discriminatory to provide an accurate definition of the identity of yeast isolates.

It is noteworthy that the majority of the 195 yeasts isolated from sausages obtained from many places in the U.K. and ingredients belonged to six genera: Candida, Cryptococcus, Debaryomyces, Pichia, Rhodotorula and Torulopsis. It was noted in the Literature Review (pp 40 - 45, Table 29) that representatives of these genera appear to be of fairly common occurrence on meat and meat products. Representatives of these genera were found also to be common contaminants of equipment and air throughout those parts of the factory in which the meat was butchered and processed. In contrast, the principal contaminant of the yeast flora in the factory's slaughter area and lairage was Tr. cutaneum. It was presumed that the radical change in the yeast flora, along what can be considered to be a continuous production line, was attributable in part to the efficacy of the washing and singeing processes, both of which are well knownto reduce the size and composition of the bacterial flora of carcasses (Dockerty et al., 1970; Snijders, 1976; Snijders and Gerats, 1976; Rashe et al., 1978; Butler et al., 1980) and in part to the failure of Tr. cutaneum to grow at chill temperatures (Table 12). The generalised distribution of the composite yeast flora in the meat processing part of the factory can be attributed to cross contamination via equipment, chopping

Table 29 Yeasts in meat and meat products.

Reference +	Commodity +	Identified with					
		Candida	Cryptococcus	Debaryomyces	Rhodotorula	Torulopsis	Trichosporon
Abbiss(1978)	Sausage	+	+		+	+	+
Ayres (1960)	Red meat	+			+	+	
Banks (1983)	Sausage	+	+	+	+	+	+
Drake (1958, 1959)	Fermented Sausage	+		+		+	+
Gardner (1971)	Bacon	+		+	+	+	
Ingram (1952)	Hams	+		+	+	+	
Lowry and Gill (1984)	Lamb	+	+				+
Mrak & Bonar (1932)	Weiner Sausage			+			
Njuko-Obi et al.(1957)	Poultry				+		

boards, knives and handling as has been shown to operate in the dissemination of bacteria (Empey and Scott, 1939; Nottingham, 1982). It is notable that members of the genera Candida, Cryptococcus, Debaryomyces, Torulopsis, Rhodotorula and Pichia were isolated from minced meats obtained from many outlets in Bath. Thus, it can be concluded from this study (Table 13) and limited literature (Table 29) that a narrow range of yeast genera occur generally on meat and meat products. Indeed, compositional differences between sausages and minced meat did not appear to affect the range of the yeasts present. Moreover, in general, the preservative, sulphite, did not influence this range although it appeared to favour the growth of the representatives of only some of the genera. Thus, sulphite appeared to favour the growth of D. hansenii and certain Candida spp, probably at the expense of Cryptococcus and Rhodotorula spp. Indeed, the presence of sulphite did not appear to influence the tolerance of the isolates of the yeast species recovered from sausage and minced meat to the preservative as judged by growth on malt extract agar containing 20, 40 and 80% bismuth sulphite agar (lab m, Table 18). The heat treatment of skinless sausages during the course of manufacture did not modify the composition of the yeast flora although the incidence of the ascosporogenous D. hansenii, the most frequently isolated yeast species from sausage and minced meat included in this survey, was reduced. This observation contrasts with the findings of Put and DeJong (1980), who concluded that the heat

resistance of ascospores was the reason for such yeasts being common contaminants of heat treated beverages. Many factors, such as the absence of D. hansenii ascospores at the time of heat treatment (Put et al., 1977) and also the solute content, a_w and particularly the pH of the environment (Juven et al., 1978; Graumlick and Stevenson, 1978), may be responsible for the observed reduced recovery of this species as a consequence of heat treatment. Thus, in general, it would appear that the yeast flora of meat and meat products is only marginally affected by ingredients and processing systems.

(2) Physiological attributes

The yeast flora from sausage and minced beef consisted mainly of non-fermentative, non-nitrate assimilating organisms capable of assimilating a wide variety of compounds (Table 14). The British fresh sausage contains a large reservoir of carbohydrate (the rusks or breadcrumbs, pp 17-19)in the form of starch (Pearson, 1970; Leads, 1979). Although only one quarter of the yeasts isolated in this study were able to assimilate starch as a sole source of carbon, the majority assimilated glucose, maltose and maltotriose. These three have been shown to occur in sausage at 7,6 and 3 mg g^{-1} respectively during storage as a consequence of the activity of amylases of meat and rusk origin (Abbiss, 1978, pp 17-19). Thus, it would seem reasonable to assume that the yeast population relies on the endogenous amylase activity of the

sausage for the conversion of starch into more easily metabolisable substrates. Extracellular amylase activity was demonstrated in broth cultures of D. hansenii, T. candida and to, a lesser extent, Cr. albidus var. albidus and P. membranaefaciens, following incubation at 4 or 22°C (Table 15). Only the first mentioned yeast generated glucose from starch probably through glucoamylase activity. It is conceivable that the sporadic occurrence of glucoamylase activity in sausages noted by Abbiss (1978) was due to D. hansenii. Indeed, this author was of the opinion that this activity was due to micro-organisms because it was not detectable in either meat or rusks. He suggested the variability may reflect a microbial mechanism by which glucoamylase was produced solely in response to a limited supply of nutrients. Under such conditions, yeast mediated amylase activity may provide a means by which additional carbon sources are released from starch.

The majority of yeasts isolates from all samples utilised lactate (ca 55% Table 14), the principal product of post-mortem glycolysis of jointed and carcass meat (Lawrie, 1974). A small proportion of isolates assimilated nucleic acid degradation products present in the meat such as ribose (ca 39%), ribitol (ca 44%, Lawrie, 1974) and also gluconate (ca 27%) which has been shown recently (Nychas, 1984) to occur in meat as a consequence of microbial metabolism. Thus, it would appear that the yeast flora are endowed with the necessary attributes to exploit the carbohydrate content of the sausage.

One third of the yeast isolates possessed lipolytic activities (Table 16) as shown by hydrolysis of Tweens in a nutrient agar. Chemical analysis of cell free supernatants of broth cultures of selected strains (Table 17) confirmed the lipolytic activities against a wide range of triglycerides (triacetate, tributyrate, tricaprionate, trioleate and olive oil) and also pork and beef fat. C. lipolytica var. lipolytica, C. zeylanoides, Cr. albidus var. albidus, D. hansenii and T. candida hydrolysed Tweens 20, 40, 60, 80 and lipases were present in culture supernatants (Table 17). Rh. rubra and P. membranaefaciens did not produce extracellular lipases or hydrolyse Tweens. The close correlation between lipolysis as shown by Tween breakdown and that demonstrated with cell free supernatants indicated that, at least for the organisms obtained from meat, the former is a useful general indicator of lipolysis and possible chemical change in sausages. Ingram (1958) considered that, in general, fat hydrolysis was the major feature of yeast spoilage. As fats constitute 10 - 15% of the sausage it represents a large reservoir of carbon and energy in the form of triglycerides. Yeast mediated lipolytic activities, presumably involving glycerol ester hydrolases, may serve to release fatty acids and glycerol from the triglycerides of pork fat thereby providing further carbon sources for the microbial population. Indeed, Brown (1977) attributed fatty acid accumulation in sausages during storage to microbial degradation of fat, a role later associated

with the yeast population by g.l.c. analysis of volatile fatty acid content in sausage (Leads, 1979).

When the Hyde powder azure (Difco, Cliffe and Law, 1982) was used, proteolytic activity could not be demonstrated in either of two broth cultures of the yeasts isolated frequently from sausage, namely C. curvata; C. lipolytica var. lipolytica; C. zeylanoides; Cr. albidus var. albidus; D. hansenii; P. membranaefaciens; Rh.rubra and T. candida. In contrast, dairy isolates (Banks pers. comm.) have been shown by the same technique to be strongly proteolytic. One possible explanation for the apparent absence of proteolytic activity could be that the high concentration of glucose in sausage elects a non proteolytic yeast flora which may then contribute to the glucose sparing affect on protein utilisation observed by Mossel and Ingram (1955). Isolates from minced meat were not studied in sufficient detail to test fully this hypothesis. Nevertheless, the absence of proteolytic activity may be a characteristic feature of yeasts in meat products.

In general, it would appear from the present study that the metabolic attributes of the yeasts of sausages are such that these organisms are, at least in theory, well adapted to grow in the commodity. Indeed, these findings suggest that this group of organisms have the potential to contribute to spoilage either directly by lipolysis of fat or indirectly by providing additional nutrients such as sugars, free fatty acids and glycerol for the spoilage bacteria. It should be stressed, however, that

sulphite, a well known inhibitor of metabolic processes (to be discussed later pp 195-200), was not included in these experiments and therefore the influence of this compound on physiological attributes needs to be assessed before any definite conclusions concerning the role of yeasts in spoilage can be drawn.

(3) Sulphite binding

It is evident from the literature on the mechanism of antimicrobial action of sulphur dioxide and its salts that the term sulphite is used routinely with no regard for the pH of the test environment. As previously mentioned (pp 7-9), pH determines the degree of ionisation of the molecule (King et al., 1981, Figure 1) and, thus, the concentration of the three possible states of the preservative in dilute solutions, sulphur dioxide, bisulphite and sulphite ions. For this reason the predominant state of the preservative in each experimental system discussed below will be referred to.

Sulphur dioxide is a well known inhibitor of yeasts (Neuberg, 1929; Rehm, 1964; Hammond and Carr, 1976). The factors especially pH, temperature, and the degree of sulphite binding, influencing the antimicrobial activity of the preservative have been discussed in detail in the Literature Review (pp 7-13).

Several mechanisms by which the preservative is transported

into the cell have been presumed. Some workers (Macris, 1972; Macris and Markakis, 1974) consider transport to be an active carrier-mediated process, whereas Stratford (1983) is of the opinion that sulphur dioxide enters the cell by diffusion. In general, inhibition of micro-organisms is considered to be the result of "multisite disruption" (Hammond and Carr, 1976; Lück, 1977) because there are many cell targets with which SO_2, HSO_3^- and SO_3^{2} can react.

Schimz and Holzer (1979) were the first to demonstrate that the ATP content of intact cells of Saccharomyces cerevisiae decreased almost immediately after addition of sulphur dioxide. The resulting damage, from the interruption of ATP dependant processes, was found to be irreversible after 60 minutes. These authors attributed such findings to enhanced utilisation of ATP as a consequence of sulphur dioxide activation of an ATP ase. Banks (1983) and Stratford (1983) both cite personal communications from Dr. D.H. Warth in which he suggested that the ionisation of "sulphite" occurred within the cell as a consequence of the higher pH (liberates protons thereby lowering the internal pH value). The release of protons would cause the membrane proton gradient to disappear and, as a consequence, the yeast would expel ATP in an attempt to re-establish the gradient. Recently, Schimz (1980) demonstrated that a concentration of ca 1 µg "sulphite" ml^{-1} inhibited the growth of S. cerevisiae in broth culture, and that this effect was increased with a decrease in the

pH of the culture medium. He found that small increases in the concentration killed the cells. In both instances cell death occurred after a lag period, the length of which appeared to be determined by the physiological status of the cells. Thus, actively growing ones survived longer than those in the stationary phase of growth.

It has been known for many years that glycerol synthesis by S. cerevisiae is enhanced by SO_2 at non-lethal levels (Neuberg and Reinforth, 1918, 1919). Indeed, SO_2 added sequentially in sublethal doses has been used to induce glycerol synthesis (Freeman and Donald, 1957). It is presumed that glycerol is formed from glyceraldehyde 3-phosphate as a result of SO_2 blockage of glycolysis (Gancedo et al., 1968). Stratford (1983) noted that the regeneration of reduced nicotinamide adenine dinucleotide (NAD^+) by ethanol dehydrogenase in anaerobically grown cells of S. cerevisiae was prevented by sulphite binding to acetaldehyde. Shortage of NAD^+ would then restrict glyceraldehyde-3-phosphate activity. The resulting accumulation of 3-phosphoglycerate would promote glycerol synthesis. Some NAD^+ would be regenerated but no net energy would be formed by this means. In contrast, Stratford (1983) was of the opinion that aerobically grown cells of this organism, in which the rate of decarboxylation of pyruvate was maintained despite binding to acetaldehyde, regenerated NAD^+ by using oxygen as an electron acceptor. It was presumed that under these circumstances SO_2

prevented ATP generation from glycolysis by acting as an uncoupler of oxidative phosphorylation or by acting by increasing the rate of ATP utilisation as previously described (p 197).

"Sulphite" is considered to be a powerful inhibitor of NAD dependent reactions (Pfleiderer et al., 1956). Indeed, inhibition of energy-yielding oxidative reactions by direct combination of sulphite with NAD is well documented (Meyerhof et al., 1938; Dupuy, 1958). Rehm (1964) was of the opinion that sulphite inhibition of respiration in yeast was a direct result of the formation of such NAD - sulphite addition complexes. He suggested that at least three steps in glycolysis of S. cerevisiae could be inhibited in this way, of these glyceraldehyde -3-phosphate → 1, 3-di-phosphoenol pyruvate was considered to be the most critical (Wallnofer and Rehm, 1965). Moreover NAD dependent steps in the T.C.A. cycle , notably malate → oxaloacetate and α oxoglutarate → succinyl coA, are thought to be inhibited by "sulphite" binding to NAD (Rehm, 1964; Wallnofer and Rehm, 1965).

Sulphite may also inhibit growth by binding to intermediates of glycolysis such as glucose, glyceraldehyde-3-phosphate, 3-phospho-dihydroxyacetone, pyruvate, acetaldehyde, oxaloacetate, 2-oxoglut-arate, thus, effectively removing them from metabolism (Hammond and Carr, 1976).

Many enzyme systems have been shown to be inhibited by sulphite *in vitro* (Pfleiderer *et al.*, 1956). Indeed, it is well established that sulphite cleaves disulphide bridges in proteins by replacing the sulphur group from the bond with cysteine or the cysteine peptides (Clark, 1932). Cleavage of enzyme disulphide bridges would lead to gross disruption of secondary and tertiary structures and more importantly loss of enzyme function (Cole, 1967).

Thiamine *in vivo* is also thought to be irreversibly cleaved by sulphite (Williams, 1935; Williams *et al.*, 1935). Cleavage of a derivative of this vitamin, thiamine pyrophosphate, which is considered to be a co-factor of microbial reactions, would lead to inhibition of all thiamine-dependent reactions (Haisman, 1974). Sulphite is considered to inhibit β. galactosidase induction of RNA and protein synthesis in *E. coli* (Robakis, 1982).

Pyridoxal phosphate (Adams, 1969), isoalloxazine (Muller and Massey, 1971; Hevesi and Bruice, 1973), glutathione (Massey and Williams, 1965) and folic acid (Vonderschmitt *et al.*, 1971) are thought to react with sulphite thus highlighting other potential targets of sulphite action on microbial metabolism. Peroxidation of lipids (Utsumi *et al.*, 1973) and also addition of sulphur dioxide to olefinic double bonds has been reported (Hammond and Carr, 1976). Keenan and Rose (1979) were of the opinion that the latter could modify the activity of yeast cell membranes.

Sulphite may be considered as a potential mutagen as it is considered to aid conversion of (in vitro) cytosine to uracil (Hayatsu et al., 1970), form addition complexes with cytosine and uracil (Shapiro et al., 1970) and to catalyse reactions of cytosine derivatives with amines to form 4-amino-cytosine (Shapiro et al., 1970). Sulphite-induced mutations have been reported in bacteriophages (Hayatsu and Miura, 1970; Summers and Drake, 1971) bacteria (Mukai et al., 1970) and yeasts (Dorange and Dupuy, 1972). It should be stressed, however, that the concentrations used in these experiments were far in excess of those found in sausage and other sulphited foods.

The generally accepted view that bound sulphite is not an antimicrobial agent (Ingram, 1948; Hammond and Carr, 1976) led (Stratford (1983) to surmise that the production of binding compounds by yeasts was a mechanism by which these organisms detoxified the preservative. Indeed, it is well established (Rehm and Wittman 1962; Reed and Peppler, 1973) that fermentative yeasts, which possess the ability to synthesise the most efficient sulphite binding compound, acetaldehyde, (Burroughs and Sparks, 1973), are more resistant to molecular sulphur dioxide than oxidative yeasts that do not.

The hypothesis of Brown (1977), that the yeast flora of sausage produced compounds which served to lower the antimicrobial activity of the preservative in sausage, was studied initially in broth cultures of the yeast species isolated most commonly from

sausage. This range of yeasts differed markedly from those used in studies of the mechanism of sulphite action discussed above.

In general, in the presence of sulphite (500 $\mu g\ ml^{-1}$) some yeasts isolated from sausage (notably D. hansenii; C. zeylanoides; T. candida and P. membranaefaciens) but not all (Cr. albidus var. albidus and Rh.rubra) produced, during the exponential phase of growth, acetaldehyde, the principal sulphur dioxide binding agent of wines and ciders (Weeks, 1969; Burroughs and Sparks, 1973). By binding to the preservative, this compound was deemed to be solely responsible for the removal of free sulphite from solution. Similarly, acetaldehyde production was demonstrated in cultures of D. hansenii and P. membranaefaciens but not Rh.Rubra derived from the NCYC. This observation suggests that this attribute was not associated with isolates from sausage only. It should be recalled (pp 94-108) that the acetaldehyde-producing yeasts D. hansenii and C. zeylanoides are those species whose incidence in sausage studied in this survey tended to be enhanced and conversely those of the non-sulphite binding species to be decreased by the presence of sulphite. Thus, is would appear that the inclusion of sulphite has the tendency to elect yeasts capable of sulphite binding. It was estimated that the concentrations of acetaldehyde attained were sufficient to account, on average, for upwards of 94% of the total sulphite. Moreover, the position of the binding equilibria

(Burroughs and Sparks, 1973) is such that almost all the available sulphite would be bound to acetaldehyde. Indeed, it is reasonable to assume that, as has been demonstrated in sulphited fruit juices (Kerp, 1904), acetaldehyde scavenged sulphite from addition complexes with carbonyls and sugars present in the media. These have lower affinities for sulphite.

As negligible concentrations of acetaldehyde were produced in the absence of sulphite, the production of this compound was obviously induced by sulphite.

Sulphur dioxide-induced acetaldehyde synthesis by S. cerevisiae and S'codes ludwigii in broth cultures has been demonstrated recently by Stratford (1983) and Morgan (1982). The mechanism by which this occurred has not been studied. Acetaldehyde synthesis by yeasts is thought to occur predominantly via a fermentative pathway (Figure 50). The acetaldehyde producing species investigated in my work, however, were non-fermentative as judged by the accepted tests. Gas production, indicative of fermentative growth, occurred however in sulphited (500 $\mu g\ ml^{-1}$) lab lemco broth. Following discussions with Professor A.H. Rose a possible explanation of these observations can be advanced (Figure 51). The strains of D. hansenii, C. zeylanoides, P. membranaefaciens and T. candida, all of which are listed by Lodder (1970) as either weakly or non-fermentative, obviously posses the necessary enzyme systems but, due to the extremely toxic nature of this compound, it inhibits the enzyme

Figure 50 Acetaldehyde synthesis in yeast.

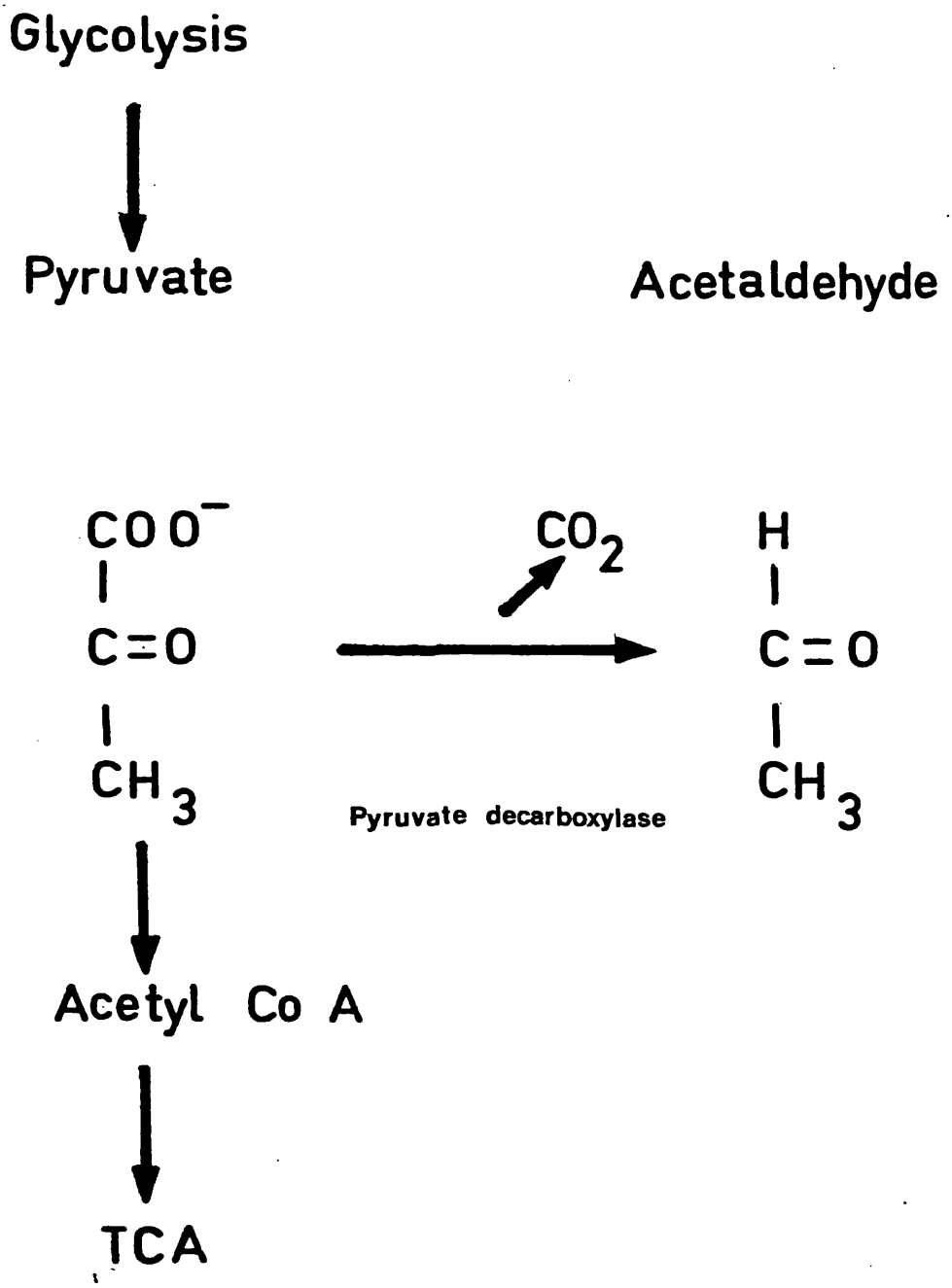
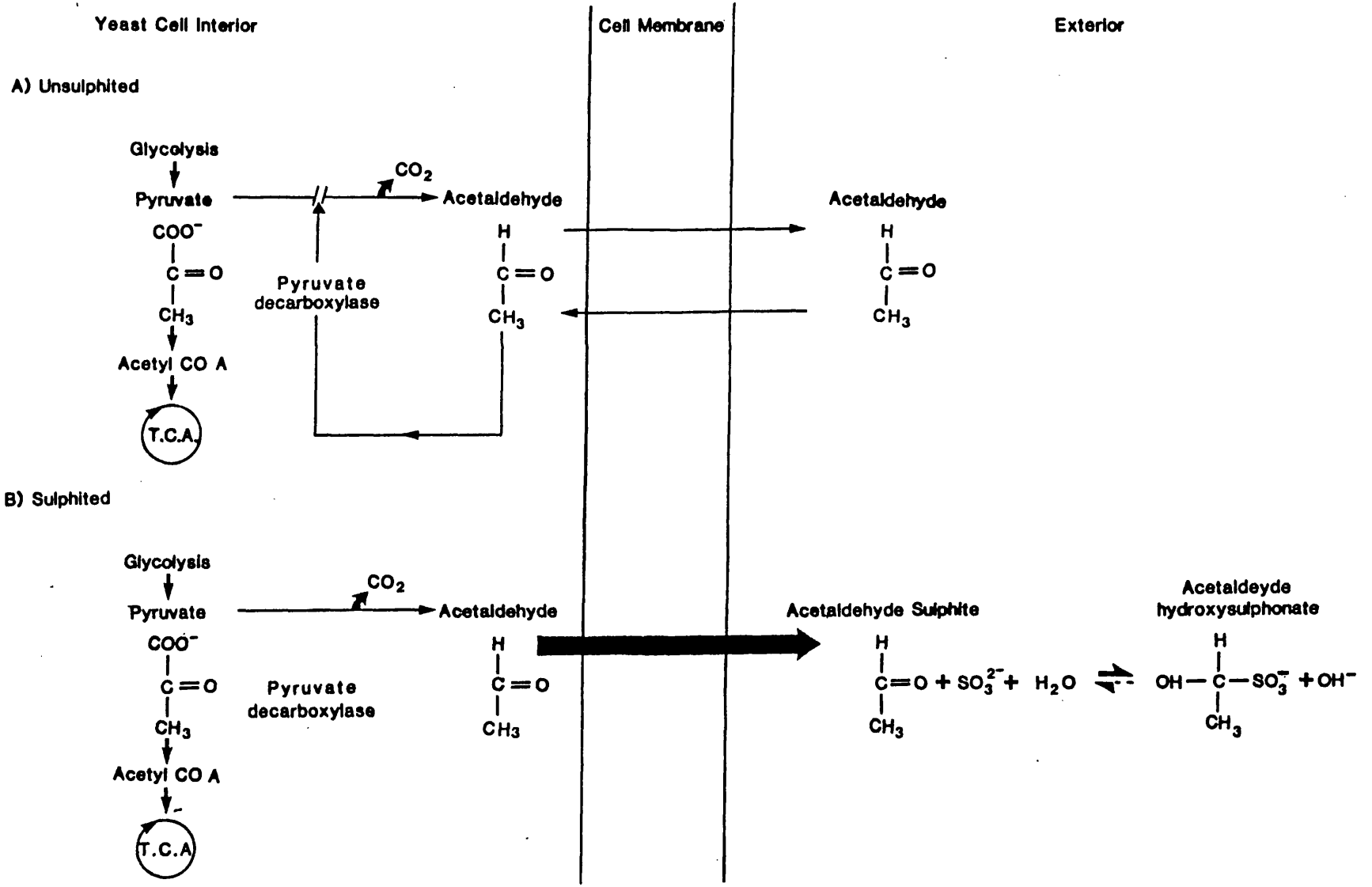


Figure 51 Proposed mechanism for sulphite-induced acetaldehyde synthesis in yeast.



pyruvate decarboxylase. If one assumes that acetaldehyde diffuses outwards across the yeast cell membrane, then the observed sulphite-induced synthesis may be attributed to sulphite binding to acetaldehyde distorting the balance between the intra and extra cellular concentrations of this substance. Acetaldehyde would then be drawn from within the cell by diffusion thereby lowering the intracellular concentrations of the compound and, as a consequence, the inhibition of acetaldehyde synthesis would cease. Theoretically, acetaldehyde synthesis would continue until the balance of intra and extra cellular concentrations was restored. That is to say, when all the available free sulphite had been removed from solution. Synthesis of acetaldehyde by such a mechanism would, however, lead to a depletion in the concentration of reduced NAD, thus restricting the flow of intermediates through glycolysis and further decarboxylation to pyruvate by curbing the activity of glyceraldehyde 3 phosphate dehydrogenase. As the rate and extent of growth of the yeasts listed above were not impeded it was presumed that Stratford's (1983) contention that NAD^+ was regenerated in aerobically grown cells of S. cerevisiae by using oxygen as an electron acceptor (pp 198-199) applied here also.

The oxidative non-sulphite binding yeasts Cr. albidus var. albidus and Rh.rubra studied in this survey were relatively unaffected by sulphite (500 $\mu g\ ml^{-1}$) in broth cultures. The mechanisms by which these organisms overcome the toxic effects

of sulphite is unclear as there is a marked absence of literature concerning the physiology of these species. Sulphite tolerance may be incurred, as suggested by Hammond and Carr (1976), by differential uptake of the preservative. Other explanations could be that, (1) the normal targets of sulphite action within the cell (pp 197-202) are insensitive to the preservative at the concentrations used in this study and (2) these organisms do not permit sulphite to enter the cell.

Minced pork belly meat, prepared for use in a large sausage factory, was used in initial investigations of sulphite binding. It harboured a pseudomonad-dominated microflora similar to that of chilled meats (Ayres, 1960). The inclusion of metabisulphite (500 μg sulphite g^{-1}) elected a flora similar to that of sausages (Dowdell and Board, 1971; Brown, 1977; Abbiss, 1978; Leads, 1979 and Banks, 1983; pp 31-36). The concentrations of free sulphite diminished rapidly following storage of the mince at 1 or 15°C. In contrast to the situation in sausage (Banks, 1983; pp 82-84), there was a rapid loss in the concentration of total sulphite and also, after an initial increase, the concentration of bound sulphite. These findings would suggest that the irreversible loss of sulphite, presumably by oxidation, is of far greater importance in pork meat than in sausage, a feature noted by Brown (1977) who reported that pork meat catalysed the oxidation of the preservative. This attribute was removed by the addition of rusks. He concluded also that emulsification (pp 3-5) which increases the extent of

cellular disruption and decreases the extent of hydration of the commodity serves to decrease the catalytic potential of the meat such that the oxidative processes leading to sulphite loss are inhibited.

Brown (1977) also noted that supplementation with rusks, which bind only small amounts of sulphite, increased the sulphite binding potential of the pork meat - supplementing the pork mince with starch (2%), the principal compound of rusks (Pearson, 1970; Abbiss, 1978) did not increase initially the content of binding agents in minced pork but did so with storage for 48 h at 15°C. These findings and those of Brown (1977) suggest that rusk and starch provide a reservoir of binding agents which can only contribute to sulphite binding as a consequence of temperature dependent activities of the meat. It is known that pork meat contains a pancreatic type as well as a salivary gland type amylase (Lawrie,1974), the activity of which have been shown in vitro to be increased by a rise in temperature. Thus, it would seem reasonable to assume that the observed increase in the content of binding agents was a result of endogenous amylase activity which converts the relatively unreactive starch to glucose and maltose, which have higher affinities for sulphite (Burroughs and Sparks, 1973). These compounds have been shown to contribute to binding in sulphite-preserved fruit juices (Ingram and Vas, 1950 a, b), ciders (Burroughs and Sparks, 1964, 1973) and wines (Weeks, 1969). Indeed, supplementing pork mince with

glucose (2%) caused an immediate increase (80 - 130 $\mu g\ g^{-1}$) in the extent of sulphite binding. It was estimated, however, that, as in sausage (Abbiss, 1978), the concentration of glucose was substantially in excess of that required to bind all the sulphite added to pork mince. Despite this, free sulphite was still available in sufficient amounts so that a characteristic microbial association (Banks, 1983) developed. Thus, it would appear that glucose is not an efficient sulphite binding agent in sausage and meat products. Brown (1977) demonstrated in experiments with a sausage slurry that the most extensive binding of sulphite was due to the micro-organisms, particularly the yeasts. I found that the rate and extent of sulphite binding in pork belly mince was appreciably higher in samples inoculated with acetaldehyde producing (pp 135-147) D.hansenii than in those inoculated with other members of the microbial association. Indeed, this activity was positively related to the size of the initial inoculum of the yeast ($r = 0.98$ and 0.92 at 1 and 15°C). When the extent of binding in sausages along with the content of binding agents were studied it was evident that the concentrations of acetaldehyde, the principal binding compound synthesised by yeasts in broth culture, were such that it could be considered to be the major sulphite-binding compound. Indeed, the concentrations of this compound and also that of sulphite binding agents in sausages obtained from retail outlets and also samples which had been stored were positively related ($r = 0.89$). It was

estimated from the binding equilibria proposed by Burroughs and Sparks (1973) that the proportion of acetaldehyde-bound sulphite increased during storage but that the concentrations of acetaldehyde produced were not sufficient to account for all the binding. Thus, it would appear that the "stripping" of already bound sulphite observed in sulphited lab lemco broths (pp 135-147) did not occur in sausage. In other words, substances present in the raw materials that bound sulphite at the time of manufacture continued to do so throughout storage. It is possible that volatilisation following extended storage was responsible for this. The occurrence of acetaldehyde concentrations far in excess of that required to bind all the preservative in 3 samples of sausages obtained from retail outlets containing exceptional concentrations of sulphite (700 - 900 $\mu g\ g^{-1}$) would suggest that such extreme conditions induce over-production of acetaldehyde.

The conclusion that acetaldehyde was of yeast origin was based on my observations in stored sausages that, as noted in yeast broth cultures (pp 135-147) the majority of acetaldehyde was produced during the exponential phase of yeast growth, the rate of synthesis being appreciably higher during storage at 15°C, when the extent of growth of the yeast populations (measured by means of doubling times) was significantly greater. These findings provide for the first time evidence to support the contention of Brown (1977) that acetaldehyde of yeast origin

was the major sulphite binding compound in sausage. This situation is akin to that observed in sulphited ciders (Burroughs and Sparks, 1964, 1973) and wines (Weeks, 1969) suggesting that this phenomenon is probably common to sulphite-preserved foods and beverages.

Such binding activities, which serve to render sulphite ineffective, raise a question over the usefulness of this compound as a preservative in commodities in which yeast growth is unrestricted. The replacement of this compound by a more efficient antimicrobial agent would be difficult, however, because sulphite is considered to contribute to the organoleptic stability of the sausage. For this reason, attention ought to be given to the use of additional antimicrobial agents for commercial use with sulphite with the objective of retarding yeast growth and ultimately the extent of sulphite binding in the British fresh sausage. Chemical preservatives such as sorbate, benzoate and paraben, which are included in a wide range of products, are generally considered to inhibit the growth of yeasts (Robach, 1980). Of these, probably only the first mentioned compound possesses the necessary characteristics, notably solubility (139_g 100 ml $^{-1}$ at 20°C), pH optima (pH 6.8; Robach, 1980) to be of use in sausage. Modified gas atmospheres containing high partial pressures of carbon dioxide are known to inhibit the growth of aerobic micro-organisms in fresh meat

(Ogilvy and Ayres, 1953; Roth and Clark, 1972; Huffman, et al., 1975). Nychas (1984) demonstrated that such atmospheres in minced meat prepared for retail retarded the rate of growth of yeasts particularly at refrigerated temperatures. Recently Banks (1983) investigated the influence of carbon dioxide (20% v/v) and sorbate (2000$\mu g\ g^{-1}$) either alone or in combination with legally permitted levels of sulphite in sausages produced on a pilot scale and stored at 4 or 10°C. He noted that both agents inhibited the rate of the growth of yeast contaminants particularly at low storage temperatures and in the presence of sulphite. More significantly, both treatments appeared to reduce the extent of binding immediately following manufacture and also the rate and extent of binding during storage at 4 and 10°C. In both cases the effect was most pronounced in samples containing carbon dioxide. Both agents enhanced the inhibitory action of the preservative such that the onset of the increase in total viable count was delayed and the subsequent rate and extent of growth of the general contaminants was substantially reduced. These observations were not evident in samples lacking sulphite and therefore it is probable that the enhancement of the antimicrobial activity of sulphite described above was a consequence of inhibition of yeast growth thereby maintaining the concentrations of free sulphite, the antimicrobial moiety of the preservative in sausage (pp 7-9).

These findings and also those presented in this study would

suggest that the control of yeast growth in such a manner would serve to extend the shelf life of the commodity. Combinations of preservatives should therefore be studied extensively with a view to their adoption for routine commercial use in the British fresh sausage and other sulphite preserved foods.

APPENDIX

APPENDIX

Names and synonyms of the yeast species isolated from sausage and minced meat.

Name proposed by Lodder (1970).	Synonyms *
Bullera	
alba †	Basionym Sporobolomyces albus
tsugae †	
Candida	
albicans †	Basionym Oidium albicans
ciferri	- Stephanoascus ciferri †
curvata	= Cryptococcus curvatus †
foliarum	Candida foliorum
humicola	= Cryptococcus humicolos †
ingens	Anamorph of Pichia humboldtii
lipolytica var. deformans	= Yarrowia lipolytica †
lipolytica var. lipolytica	Anamorph of Yarrowia lipolytica
mesenterica	Basionym Pseudomonilia mesenterica
ravautii †	= Candida catenulata †
rugosa †	Basionym Mycotorula rugosa
sake †	Basionym Eutorulopsis sake
silvae †	
valida †	Anamorph of Pichia membranaefaciens
vini †	
zeylanoides †	Basionym Monilia zeylanoides

Cryptococcus	
albidus var aerius	= Cryptococuss albidus +
albidus var albidus +	Basionym Torula albidus
albidus var diffluens	- Cryptococcus ablidus +
dimennae +	
gastricus +	
hungaricus +	Basionym Dioszegia hungaria
laurentii var flavescens	- Cryptococcus laurentii
laurentii var laurentii +	Basionym Torula laurentii
laurentii var magnus	= Cryptococcus magnus
macerans +	Basionym Rhodotorula macerans
skinnerii +	
uniguttulatus +	Anamorph of Filobasidium uniguttulatus
Debaryomyces	
hansenii +	Basionym Saccharomyces hansenii
marama +	
Hansenula	
polymorpha +	
Leucosporidium	
capsuligenum	- Filobasidium capsuligenum +
scottii +	
Pichia	
etchellsii +	
media	

Pichia	
membranaefaciens +	
vini var melibiosi	- Pichia carsonii +
vini var vini	- Pichia carsonii +
Rhodotorula	
glutinis var glutinis +	
graminis +	
marina +	
minuta var minuta +	Basionym Torula minuta
pallida +	
rubra	Rhodotorula muciliginosa +
Torulopsis	
candida	= Debaryomyces hansenii
domerqii	= Wickerhamiella domerqiae
inconspicua	= Candida inconspicua +
ingeniosa	= Rhodotorula ingeniosa +
norvegica	= Candida norvegica +
vanderwaltii	= Candida vanderwaltii +
versatillis	= Candida versatillis +
Trichosporon	
cutaneum	- Trichosporon beigelii +

KEY

* Adapted from Barnett et al. (1983).

+ Currently accepted species.

\- is synonymous with but does not share the type strain.

= is obligatery synonymous with and shares the same type strain.

Throughout this thesis Saccharomyces, Debaryomyces and Leucosporidium have been abbreviated as S., D. and L. respectively. It should be noted that the abbreviations for these genera should be Sacch., Deb. and Leu. as given by Lodder (1970).

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